



Metabolism and inflammation: implications for traumatic brain injury therapeutics

Journal:	<i>Expert Review of Neurotherapeutics</i>
Manuscript ID	ERN-2018-0136.R1
Manuscript Type:	Review (invited)
Keywords:	Inflammation, Metabolic Dysfunction, Metabolism, Supplementation, Traumatic Brain Injury, TBI, Treatment, TCA cycle, Glycolysis, Lactate/Pyruvate Ratio

SCHOLARONE™
Manuscripts

EXPERT REVIEW OF NEUROTHERAPEUTICS

Revised Manuscript for Submission
Changes in red text

Metabolism and inflammation: Implications for traumatic brain injury therapeutics

ABSTRACT

Introduction: Traumatic Brain Injury (TBI) is a leading cause of death and disability in young people, affecting 69 million people annually, worldwide. The initial trauma disrupts brain homeostasis resulting in metabolic dysfunction and an inflammatory cascade, which can then promote further neurodegenerative effects for months or years, as a ‘secondary’ injury. Effective targeting of the cerebral inflammatory system is challenging due to its complex, pleiotropic nature. Cell metabolism plays a key role in many diseases, and increased disturbance in the TBI metabolic state is associated with poorer patient outcomes. Investigating critical metabolic pathways, and their links to inflammation, can potentially identify supplements which alter the brain’s long-term response to TBI and improve recovery.

Areas covered: The authors provide an overview of literature on metabolism and inflammation following TBI, and from relevant pre-clinical and clinical studies, propose therapeutic strategies.

Expert commentary: There is still no specific active drug treatment for TBI. Changes in metabolic and inflammatory states have been reported after TBI and appear linked. Understanding more about abnormal cerebral metabolism following TBI, and its relationship with cerebral inflammation, will provide essential information for designing therapies, with implications for neurocritical care and for alleviating long-term disability and neurodegeneration in post-TBI patients.

KEYWORDS: Inflammation, Lactate Pyruvate Ratio (LPR), Metabolic Dysfunction, Metabolism, Supplementation, Traumatic brain injury (TBI).

1.0 Traumatic brain injury

Traumatic Brain Injury (TBI) is the leading cause of death and disability for those under 40 years of age in the developed world [1,2]. While an estimated 69 million people are affected worldwide every year [3], there has been significant progress in the last century to combat the extent of such injuries. Public policies such as road safety measures and occupational health and safety regulations can decrease the incidence of TBI, and advanced neurocritical care has improved individual patient management. Multi-modality monitoring is one of such advancements which enables real-time observation of cerebral pressure and oxygenation. Clinicians can then attempt to resolve disturbances in these parameters of the brain using surgical and medical interventions. Despite the progress in these areas and our improved understanding of the brain in recent decades, there is still no standard effective drug treatment for TBI. There is a great opportunity for such treatment to mitigate the extent of tissue damage and cell death, however many clinical and pre-clinical trials that have seemed promising in earlier stages have failed to show significant benefit when implemented in patients [4]. This may be due to a number of things, including issues with animal model to human translation, lack of trial optimisation in humans, the heterogeneous nature of the injuries, shortcomings of the Glasgow Outcome Scale (GOS) in assessing smaller improvements, and the complexity of targeting the inflammatory system due to the pleiotropic nature of its components [5].

Initial or 'primary' trauma of the brain is caused by impact and physical movement of the brain, resulting in damage of blood vessels, damage to cell axons, shearing of tissue, and swelling. The 'secondary' injury of continued degeneration, however, is less well understood and can vary vastly between patients with similar injuries. TBI is a heterogeneous disease ranging in severity; this review is focussed on severe TBI in the context of neurocritical care. Recent studies have highlighted the high occurrence of chronic traumatic encephalopathy in players of contact sports, and how multiple mild head injury events can lead to chronic traumatic encephalopathy with dementia and Parkinson's-like symptoms [6]. Mild TBI and multi-injury concussive brain injury patients differ from severe TBI patients in terms of pathology [7,8], inflammatory activation levels [9], and brain metabolic changes [10]. Caution and additional research must therefore be taken when applying treatments proposed for single-injury severe TBI patients in this review to other forms of TBI.

In the days following severe TBI, we see a prominent inflammatory cascade in the majority of patients [11], with subsets of patients experiencing a chronic inflammation for many months or even years post-injury, accompanied by cognitive neurodegeneration [12]. Mitochondrial dysfunction is also a hallmark of both the acute and post-acute stages of severe TBI, with changes in mitochondrial morphology [13], accumulation of mitochondria at injury sites, altered levels of metabolites and overall mitochondrial function [14,15]. There is increasing evidence that these two phenomena of inflammation and mitochondrial metabolism have significant cross-talk and impact upon one another in multiple other disease states of

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

the brain, including multiple sclerosis [16], Parkinson’s [17], Alzheimer’s [18,19], and amyotrophic lateral sclerosis [20]. This relationship also warrants investigation in TBI, and any mechanistic links between metabolism and inflammation may be key for dual-acting therapies which can ameliorate the negative effects of both pathways.

This review will outline the critical roles and interaction of metabolism and inflammation in TBI, highlighting the implications of these interactions for potential therapeutic strategies. As this is an emerging field of interest, evidence of such interaction between the two processes in several disease models will be presented, along with the case for further research into the important relationship.

2.0 Brain Metabolism

2.1. Fundamentals of metabolism

The brain requires around 20% of the body’s total energy output to send electrical signals and maintain homeostatic function. Cellular metabolism provides the energy that underlies all brain activity by processing substrates into ATP. Glucose is the main source of fuel for energy production in the brain, but the mechanism of its consumption varies in different environmental conditions and between different cell types, with downstream substrates such as lactate purported to play a key role [21].

Glycolysis, the initial breakdown of glucose molecules, produces a low yield of 2 molecules of ATP per molecule of glucose, and 2 pyruvate molecules, which can subsequently be used for further energy production (Figure 1). After glycolysis, the pyruvate molecules can go on to be converted to lactate with no additional ATP produced, or used in the high yield oxidative phosphorylation pathway, through the tricarboxylic acid (TCA) cycle and electron transport chain (ETC). The energy yield per molecule of glucose metabolised fully to CO₂ (by the combination of glycolysis, NADH shuttling and mitochondrial respiration) is theoretically 36–38 molecules of ATP, but the actual yield is considered somewhat lower [22,23].

The pentose phosphate pathway (PPP) is yet another potential fate of a glucose molecule, diverging from initial glycolysis steps, which produces no ATP but does produce molecules for nucleic acid sequences. The PPP is a complex biosynthetic network constituting a detour around several steps of glycolysis, generating many species including lactate and does not involve molecular oxygen. Its main function can be regarded as sacrificing some of the cells’ supply of glucose molecules, which might otherwise have been used for ATP synthesis, for the sake of generating more reducing power (NADPH) and the ability to protect, repair, or build cells.

Another energetic pathway theory which is gaining in acceptance (although still debated) is the astrocyte-neuron lactate shuttle (ANLS) hypothesis [24,25]. In the ANLS theory, glycolysis - the conversion of glucose to pyruvate - occurs predominantly in astrocytes, followed by the subsequent conversion of pyruvate to

lactate by lactate dehydrogenase (LDH). The lactate is exported from astrocytes then transported by monocarboxylate transport proteins (MCT) into neurons where it can be oxidized back to pyruvate for entry into the TCA cycle. The astrocyte-neuron lactate shuttle (ANLS) is depicted schematically in Figure 2 and explained further in the legend [26].

The advantages of this transfer of lactate include the ability to maintain brain function by supporting neurons at the energetic cost of the astrocytes. Neurons, for example, have reduced expression of enzymes that detoxify by-products of glycolysis (glyoxase 1 and 2) compared to astrocytes [26]. Also, in comparison to neurons, astrocytes have lower activity of pyruvate dehydrogenase, which processes pyruvate for use in the TCA cycle [26], and a higher concentration of lactate (considered as a reservoir) [27,28]. Further evidence to support specific cell type metabolic substrate preferences is detailed in neocortical cell culture models [29] and in positive implications from small studies of human patients [30]. However, findings of a kinetic modelling study in rats using labelled glucose [31] favoured an “independent” model in which neurons and astrocytes take up and oxidise glucose according to their respective energy needs, rather than the ANLS model. It still remains to be shown directly that there is net transfer of lactate from glia to neurons *in vivo*, nevertheless it is important to consider the potential energetic impacts of such processes in our evolving understanding of brain metabolism.

Whether glucose or lactate molecules are the primary source of fuel for neuronal cells, maintaining optimum energetic functioning of the brain can thus be achieved by utilising multiple metabolic substrates. The evidence of flexibility in the uptake and use of such molecules highlights a wider range of potential targets for energetic supplementation. Optimal energy processing in both homeostasis and injury is therefore an important aspect for further research as it may assist in recovery.

2.2 Metabolism in traumatic brain injury

Distinct changes in brain metabolism become apparent when brain cells are stressed by TBI [32]. Metabolic indicators of the glycolysis pathway are increased, as well as the presence of extracellular lactate, producing a high extracellular lactate to pyruvate ratio (LPR) in some TBI patients. LPR is often used as an indicator of the brain's overall metabolic state in TBI, with a high LPR reflecting mitochondrial dysfunction or a lack of oxygen supply [15]. Increased lactate production from pyruvate may also reduce the total pyruvate available for mitochondrial metabolism, and as such reduce overall ATP production.

Prior to surgical and medical interventions, a severe head injury or insult would likely cause a lack of oxygen to brain cells – hypoxia, so this switch to glycolysis may simply be an evolutionary coping mechanism, to ensure that energy production can still occur. Glycolysis and pyruvate to lactate conversion occurs without the need for

oxygen, unlike mitochondrial energy processing, and as such, cerebral hypoxia in TBI patients is also accompanied by an increase in glutamate and lactate levels [33].

The pentose phosphate pathway (PPP) [34] also does not require oxygen and may be another source of increased lactate post-TBI. In a recent small study, several TBI patients had PPP-derived lactate elevation above “normal” (non-TBI) brain ranges, correlating with decreasing brain tissue oxygen concentrations, indicating shifting glucose metabolism from glycolysis towards PPP (although with glycolysis remaining dominant) [34].

These perturbation in brain metabolism however can still occur in TBI patients even when access to oxygen is well conserved by clinical management strategies. Despite modern neurocritical care supplying the brain with seemingly adequate levels of oxygen and nutrients (e.g. glucose), the injured brain cannot always use the fuels it receives. Such patients suffering metabolic dysfunction (characterised by a high LPR) had significantly poorer clinical outcomes in a large study (223 patients) [15]. The divergence from normal metabolic function despite adequate oxygen, may imply that the change in metabolism may be associated with additional functions than just that of producing energy, such as involvement in the other key mechanisms which cope with injury, like inflammation.

Reliance on the glycolysis pathway for energy production in TBI may also be due to damage sustained by the mitochondria [35]. Complex I of the mitochondrial electron transport chain is thought to be a likely site of impairment or alteration in activity in both TBI [36] and neurodegenerative disorders such as Parkinson’s [37], multiple sclerosis [38] and amyotrophic lateral sclerosis [39,40]. It has been demonstrated in animal models that the function of complex I decreases with age, and that it is particularly vulnerable to reactive oxygen species (ROS) which are produced adjacent to the complex [41]. The purported cause of this vulnerability is credited to the large proportion of mitochondrial rather than nuclear DNA subunits encoding complex I [42]. It is well established that mitochondrial DNA functionality decreases with age in various rodent tissues [43], accompanied by an increase in mitochondrial ROS production [41].

The fragile complex 1 may be too damaged post-TBI to utilize metabolic substrates, causing the observed extracellular accumulation and high LPR in patients [44]. Targeted supplementation of the brain’s mitochondria for rescuing the TCA cycle and sites of damage are therefore a prospective avenue for therapeutics. Recovering metabolic function may also contribute towards the whole brain response towards more reparative mechanisms such as inflammation, as detailed further in section 4.

3.0 Inflammation in TBI

The mechanism by which the force and impact of a TBI stimulates cells to initiate inflammatory signals is not fully understood. The trauma results in cellular debris and Disease Associated Molecular Patterns (DAMPs) including free DNA, RNA and alarmins such as HMGB1[45,46]. This begins the sequence of the inflammatory cascade, which activates and recruits both resident and peripheral immune cells. The inflammatory cascade initiates for a number of beneficial functions, including the phagocytosis and clearing of dead cells, the isolation of healthy brain area by 'walls' of microglia and astrocytes, and protection against infection [47]. However, persistent or unregulated inflammation can inflict 'secondary insults' on the brain, and it is difficult to detect at what point this inflammation becomes maladaptive [48]. Inflammatory processes in combination with a cytotoxic environment may lead to the excessive pruning of still-functioning synapses and engulfing of viable cells. This chronic level of inflammation and activated immune cells, which have been identified up to 17 years post-injury in patients, may play a significant role in the decrease of white matter density and corresponding reduction in cognitive ability [12,49].

There is an opportunity to intervene with treatments to limit this chronic inflammatory process, however, it is difficult differentiate the beneficial inflammation from the harmful. Inflammation in the brain post-injury could potentially be modulated by the use of targeted anti-inflammatory treatments (e.g. IL-1ra), as these may reduce the negative effects of this cascade [50]. However, a refined approach is required to maintain the beneficial aspects of inflammation such as repair and promotion cell survival [11], as anti-inflammatory therapies may therefore also impact on these restorative processes.

In ideal circumstances acute inflammation in the brain is self-resolving, yet the exact processes that govern the cascade or how to 'switch' from encouraging cell apoptosis, clearance and stress signals, to promoting cell survival and repair *in vivo* remain uncharacterised. Thelin et al. [51] have further described the complexity of the post-TBI environment, detailing the interactions between cell types and highlighting key signalling molecules, shown schematically in Figure 3 and explained in the legend. The complement system, for example, may contribute to neurodegeneration in TBI, as it enhances the inflammatory cascade. Complement, (for example C1q, C3b, C3d, C5-9) is increased in the brain post-injury and in microglia specifically – evidence has shown that this increase may influence their activation state, stimulate accumulation of inflammatory cells and formation of brain edemas and further neuronal damage [52,53]. The complement system therefore provides another avenue for exploration into inhibiting exacerbation of chronic inflammation, and research into this area is promising for future potential therapies, as detailed further in the review by Hammand et al [54]. In addition to complement, a recent study of neurogenic inflammation mediator- substance P is also an important peptide neurotransmitter to note in TBI, and is further detailed in a review by Vink et al. [55].

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

It has been established in rodent models of TBI that monocytes and other inflammatory cells such as neutrophils enter the brain via the bloodstream [56-58], however the literature is currently lacking in definitive confirmation of this process in human TBI patients. The recent discovery of microscopic ‘tunnels’ from both mouse and human skull bone marrow to the dura and meninges however illuminated additional avenues through which these inflammatory cells may invade the brain [59].

The level of blood brain barrier (BBB) dysfunction and the role it plays in regulating this infiltration is likely to differ with severity of injury, and as such is difficult to study in humans. Csuka et al. demonstrated that BBB dysfunction was independent of key cytokine levels, comparing markers of BBB dysfunction and IL-10 levels in TBI patient cerebral spinal fluid (CSF) samples [60]. While in rodent models, Semple et al. found that deficiency of a chemokine receptor, CXCR2 was able to attenuate neutrophil infiltration [61]. Invading inflammatory cells from the peripheral nervous system could be vital to recovery post-insult, or to the perpetuation of a chronic inflammatory state. Further research into the roles of these cells in injury would be of great benefit to our overall understanding of the intricate relationship between BBB functionality and inflammation in the brain.

3.1 Cytokines and glia

Cytokines and chemokines comprise a broad range of small cell signalling proteins that are autocrine and paracrine regulators of inflammation in the brain. They are released from activated, stressed or dying cells in order to promote survival, breakdown cellular membranes, recruit other cells and mark them for repair or phagocytosis [62]. Although cytokines are often classified as pro- or anti-inflammatory, many have been found to be involved in opposing roles, depending on the context of their environment and timing after injury [63].

The levels of numerous cytokines and chemokines found in TBI patients have been characterised in the days after injury in microdialysates. These studies have highlighted how specific signalling proteins can be associated with aspects of the timing and nature of the inflammatory state of the brain [11]. High IL-1 β and TNF α are typically associated with the first 24 hours post-injury, related to pro-inflammatory mechanisms including apoptosis in many disease models [64-66], while high IL-10, and later IL-4 can be associated with the latter stages and are known for their roles in repair and promoting cell survival [63]. Also of note is IL-6, an signalling molecule which appears at high concentrations in the acute phase of injury, acting as a biomarker of inflammation [67].

Microglia are the resident inflammatory cells in the brain and are a key source and regulator of the cytokine cascade. When activated *in vitro*, they can be seen to

produce profiles of cytokines which are also often classified as pro- or anti-inflammatory, namely the M1 and M2 phenotypes [68]. While this characterisation has been criticised for its simplicity in describing a complex phenomenon [69], and proposals have been made for revisions and alterations to the paradigm [70], they are nonetheless useful terms when attempting to understand and refer to the ways in which activated microglia and their cytokine profile can be associated with reparative, or destructive and phagocytic processes.

In a number of pathological conditions, such as neurodegenerative disorders, both M1 and M2 type macrophages have been found to coexist and also form other unique phenotypes, and it is likely that most cells sit on a spectrum of activation as opposed to these extreme polarised states [70,71]. It can also be difficult to distinguish infiltrating monocyte derived macrophages from activated microglia in the brain. Levels of 'CD' antigens such as CD11b and CD45 [72,73] have been used in multiple publications to differentiate these cell types in the past two decades, however newer stains and labelling systems such as TMEM119, a specific marker for microglia [74,75] are proving valuable. The impact of these infiltrating vs resident cells and their inherent neuroprotective or pro-inflammatory role are still being debated, and again can change dependant on the severity of injury [76-78].

Astrocytes have similarly been assigned A1 and A2 designations of activation in recent studies, denoting states of activation in these cells [79]. Their activation is closely linked to that of microglia; their role in TBI has been found to be important for tissue protection in mild TBI, but not severe TBI [80], and more research is required to understand their role in this specific disease context.

Oligodendrocytes also play a key role post-TBI in remyelination and repair of damaged axons, however excessive myelination by dysregulated cells may also lead to further damage [81,82]. Direct mechanistic links of oligodendrocytes to the inflammatory cascade however are not well defined, although some links have been made to metabolic dysfunction in the overall Wallerian degeneration of axons.

4. Synergistic inflammation and metabolic response

The relationship between inflammation and metabolism in TBI remains largely unexplored but may have substantial implications for treatment, given that metabolism plays a significant role in encouraging the reparative mechanisms of the brain.

Macrophages and other myeloid cells, as previously described in their involvement in the inflammatory response, have several metabolic characteristics which distinguish the M1 and M2 phenotypes *in vitro*. When these non-CNS macrophages are activated into classical M1 or alternative M2 states by LPS and inflammatory cytokines, there is a measured change in metabolism [83], including an increased

glucose consumption and lactate production [84,85]. Blocking oxidative metabolism in these cells also blocks the M2 phenotype, and drives the macrophage into an M1 state. Similarly, forcing oxidative metabolism in an M1 macrophage potentiates the M2 phenotype [83].

The hypothesis for how the change in metabolism affects the inflammatory state, is via increased TCA cycle intermediates like succinate and malate. These intermediates are responsible for regulating HIF1 α , which drives the sustained production of the pro-inflammatory cytokine IL-1 β . Therefore, the change in metabolism and build-up of such intermediates caused more 'pro-inflammatory' cytokine IL-1 β , and therefore an M1-like state.

Levels of oxygen are also theorised to play an important role in HIF α regulation, potentially in a similar way by blocking mitochondrial respiration and causing similar build-up of intermediates. Therefore hypoxia-like environments, where mitochondrial dysfunction occurs as in TBI, may similarly drive a M1 pro-inflammatory phenotype in infiltrating macrophages [83]. Microglia, as the resident macrophage cells of the brain also respond similarly to such metabolic impairments. The inhibition of mitochondrial respiratory chain **complex I** in microglia by the compound rotenone led to production of mitochondrial ROS and TNF α [86,87].

The separation of neuronal and glial cultures is important for investigation of metabolic and inflammatory pathways, as the interaction between different cell types in response to stress is complex. For example, neuronal death caused by addition of mitochondrial inhibitors to cell cultures was not observed unless microglia [88], or mixed glia [89] were also present in the culture. Gao et al attributed this phenomenon to the release of NADPH oxidase-derived superoxide from activated microglia [89], while Mount et al found that antibodies neutralizing the cytokine IFN- γ (produced by the microglia) improved neuronal survival of this mitochondrial stress [88]. Phagocytic activity of microglia was also found to be increased by mitochondrial inhibitors causing further neuronal loss in mixed neuronal/glia cultures [90]. Astrocytes also have different roles and associated response to stress and cytokines [91] and may also warrant individual investigation. Mixed cell cultures of neurons and glia are therefore necessary in order to gain a 'whole brain' perspective, while individual cell types in culture can elucidate cell-specific mechanisms.

Further evidence detailing the relationship between inflammation and metabolism is found in several disease models. Succinate produced by macrophages in an arthritis cell culture model exacerbated the inflammatory response, resulting in increased inflammatory cytokine IL-1 β [92]. In atherosclerosis mouse models, pre-treatment of macrophages with mitochondrial inhibitors abolished the anti-inflammatory effects of cytokine IL-4. This indicates that anti-inflammatory mechanisms may be modulated by metabolism. However, inhibition of mitochondrial respiration had no effect on

inflammatory activation of macrophages by proinflammatory cytokine IFN- γ and bacterial inflammatory response generator LPS [85].

More recently, in a Parkinson's disease model, transmembrane protein 173 or 'stimulator of interferon genes' (STING) was identified as the key signalling molecule activated during mitochondrial stress [17]. It also indicated the importance of proteins PINK1 and parkin, which aid in removing damaged mitochondria, in order to prevent this STING activation.

The relationship between inflammation and metabolism therefore has a strong evidence base and holds great potential for manipulation to improve injury and disease states.

5. Therapeutic avenues for TBI

5.1 Standard treatment and monitoring

Despite the progress in research in TBI, there is still no standard effective drug treatment for TBI which addresses metabolic dysfunction or the inflammatory cascade. Patients receive varying levels of other treatments and medication in accordance with their condition, for example antibiotics for pneumonia or infections, which may also impact on both their central and peripheral inflammatory state.

Multi-modality monitoring of patients enables real-time observation and intervention of disturbances in brain qualities such as pressure and oxygenation, managed both medically and surgically. Cerebral microdialysis also allows monitoring of brain glucose levels which can be controlled, as well as lactate and pyruvate as detailed further in Section 5.3, for indication of metabolic dysfunction. Monitoring of a number of biochemical compounds including cytokines have been investigated as potential biomarkers for brain injury and have been sparsely incorporated into clinical practice [11,93,94]. Ongoing biomarker analysis can assist in detection of secondary harmful events, however is not currently used for targeting therapeutics.

The majority of past TBI clinical drug studies focused on more multifunctional global brain approaches to influences hormones and steroids, including corticosteroids [95], progesterone [96,97], citicoline [98], magnesium sulphate [99], and statins [100] with limited success. This may not be due solely to poor efficacy of the therapies, as with the anti-inflammatory therapies mentioned below in 5.2, but instead could be related to multiple factors such as issues with animal model to human translation, lack of trial optimisation in humans, the heterogeneous nature of TBIs, and difficulty in assessing minor improvements in patient outcome. Some illustrative studies of previous anti-inflammatory and metabolic therapeutics for TBI are summarised in Supplementary Table 1.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

5.2 Anti-inflammatory therapies

Anti-inflammatory therapies have been extensively studied in TBI, however have yet to demonstrate definitive clinical benefit. Wide ranging anti-inflammatory agents have been repurposed for studies in TBI, with many of the aforementioned multifunctional therapies in 5.1 and Supplementary Table 1, also having a potential anti-inflammatory effect. The broad immunosuppressant minocycline has been tested with limited success in rodent models [101], phase II human trials of spinal cord injury [102], and recently in TBI patients [103]. In Scott et al. 2018, minocycline treatment managed to reduce markers indicative of microglial activation, however also increased markers of neurodegeneration in TBI patients. Common anti-inflammatory therapeutic ibuprofen caused no significant effects in clinical studies [104] and deteriorated cognitive outcomes in rodent models of TBI [105].

Targeted anti-inflammatory therapies are also being explored in TBI. These inhibit the action of specific cytokines using antibodies and synthetic receptor antagonists. Tumour necrosis factor (TNF), inhibition has shown improvements across multiple clinical domains following treatment of both stroke and TBI patients in a large cohort study [106]. This study, however, had several limitations and a small TBI cohort, and hence a randomized, placebo-controlled trial is necessary to further characterise this outcome. The inhibition of cytokines IL-1 α and IL-1 β have similarly been explored, due to their significant ‘pro-inflammatory’ effects with success in pre-clinical rodent models of TBI [107,108]. IL-1ra, the naturally occurring inhibitor of IL-1 α and IL-1 β , when given subcutaneously also reduced markers of peripheral inflammation in subarachnoid haemorrhage patients [109]. Evidence of IL-1ra relationship with TBI patient outcome was established in a small study of 15 patients by Hutchinson et al. 2007, in which higher endogenous IL-1ra levels correlated to favourable outcomes [110]. Supplementing IL-1ra levels in the brain with Anakinra (recombinant IL-1ra) has also proven to be safe in TBI patients, however, initial findings suggest that it produces an increased ‘pro-inflammatory’ or ‘M1’ cytokine profile compared to untreated patients [50,111]. These effects will be further explored in a dose optimisation study of recombinant IL-1ra in a placebo controlled randomised trial by Helmy et al. (estimated completion 2023). The extent of the numerous novel pre-clinical anti-inflammatory studies are beyond the scope of this review, however is well detailed in other publications [112-114].

Nonspecific anti-inflammatories may be able to reduce the negative effects of an overwhelming cytotoxic cascade, however, they may also dampen repair and cell survival mechanisms. Equally, it can be difficult to produce a measurable effect in humans when targeting only a single cytokine or chemokine, as they act synergistically, and it can be difficult to tease out specific cytokine functions within a complex system [115]. Antibodies or increase of specific cytokines could also potentially trigger the opposite effect to that intended, depending on dose, timing,

disease context and environmental factors. For example, cells may have their own feedback loop for limiting further transcription and production of cytokines [116,117], which anti-inflammatory therapies may interrupt.

Despite their targeting limitations, monitoring the effect that any potential TBI treatments may have on these signalling proteins is an important feature to investigate. As this review details, one potential avenue for anti-inflammatory action is through specific metabolic targeting as a dual action therapy.

5.3 Metabolic Supplementation

The brain's metabolic status post-TBI has been studied in great detail with spectral analysis techniques used noninvasively and also in the analysis of patient samples such as serum, CSF and microdialysis fluid. These techniques include; magnetic resonance spectroscopy (MRS) [118], mass spectrometry [119,120], and nuclear magnetic resonance (NMR) with both unlabelled [121] and ^{13}C labelled substrates [34,122]. The results of these studies highlight potential metabolic pathways which could be targeted by supplementation to improve brain energy status.

There is promising evidence currently emerging for metabolic supplementation, and its reinforcement of the 'normal' (non-trauma) homeostasis of the brain [123]. The impact of these substances on the inflammatory cascade, however, is yet to be demonstrated in most cases. Studies need to further investigate whether these agents will aid an overactive immune system perturbing the brain or encourage a more reparative environment.

5.3.1 Glucose

Brain glucose is of significant importance post-TBI, with patients often presenting with cerebral extracellular glucose levels outside the normal range, correlating with worse outcomes. High glucose levels identified in patient serum were associated poorer outcomes in patients [124] as were persistent low extracellular glucose levels in brain microdialysates [125]. Brain glucose is directly influenced by blood glucose levels and glutamate pathogenicity increases when blood glucose levels are low [126]. It is important to note, that glucose is of utmost importance for overall brain metabolism, and although additional substrates we are suggesting for supplementation may assist in brain metabolism in the acute phase post-TBI, glucose levels must first be corrected if at abnormal levels. The addition of glucose as metabolic rescue agent is therefore a critical factor in patient outcome, and many centres use glucose control in their standard TBI protocols, aiming for blood glucose levels of 4-7 mM [127]. However, controlling glucose levels too tightly in patients has

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

also been found to have no benefits in terms of decreased mortality [128] and increased incidences of metabolic crisis [129,130].

5.3.2 SuccinateSuccinate is another energetic molecule which has been explored as a therapeutic supplement in TBI. It acts at complex II of the Electron Transport Chain (ETC), in the mitochondria, located downstream potential sites of damage such as at complex I as previously described in 2.2. Succinate is converted into fumarate by the enzyme succinate dehydrogenase, as an integral part of the TCA cycle. Succinate, the anion of succinic acid, has multiple biological roles as a metabolic intermediate; as part of the production of ATP and as a signalling molecule of the cellular metabolic state [131].

It has been shown that succinate supplementation improves TBI brain chemistry, in a mixed glial model, indicated by biomarkers and reduced the LPR after exposure to stress by rotenone [132], highlighting its potential for use in TBI treatment. There is evidence to suggest, however, that succinate can have negative effects in hypoxic conditions, where it can build up in tissue, leading to the production of excessive reactive oxygen species (ROS) when oxygen is returned [131,133]. These studies however often used artificial dimethyl or diethyl succinate which is more cell permeable and may have further implications beyond that of natural metabolic utilisation, unlike the disodium salt. Ischemia reperfusion-induced injuries are also less common in TBI patients with modern neurocritical care that includes adequately managed brain oxygen supply as was the case in patients in a clinical study of succinate [123], and as such this succinate build up would be unlikely to occur.

Areas to be carefully considered during implementation of succinate as a therapeutic supplement therefore include ensuring adequate tissue oxygenation and reviewing the implications of using artificial features in such as methyl- and ethyl- groups on such molecules. Preliminary studies of disodium succinate use in TBI patients have provided a promising outlook for its use in delivery via microdialysis catheter, demonstrating effectiveness in lowering of the LPR [123] and improving the NADH/NAD⁺ redox state of the brain [134]. In a patient with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes), oral succinate therapy (6 g/day, for more than 30 months) was associated with freedom from the stroke-like episodes and convulsions that had afflicted this patient prior to succinate therapy [135].

Succinate's effect on inflammation, however, requires further investigation as in mice and *in vitro* experiments succinate enhanced pro-inflammatory IL-1 β production [131]. Succinate oxidation and subsequent ROS generation has also been linked to 'pro-inflammatory' macrophage activation [136]. However, in a recent paper using neural stem cells, when succinate was released from mononuclear phagocytes, it

initiated a chain of signalling resulting in anti-inflammatory effects [137], again highlighting the importance of context when assessing such complex systems.

5.3.3 Lactate

Mounting evidence indicates that lactate may be an efficient energy substrate for neurons and contribute to maintaining synaptic transmission, particularly during periods of intense activity [138] via the ANLS as previously described in 2.1. Its use as a supplement in TBI has been explored in both animal [139] and human models [27], although warrants additional clinical study in this context. The mechanism of lactate as a signalling molecule has also been explored with several different functionalities identified in depolarisation, currents and action potential activity [140]. Recent studies have also highlighted additional roles relevant to TBI, finding that L-lactate supplementation of cell culture model medium increased mRNA expression of genes regulating synaptic plasticity and neuroprotection [141].

High concentrations of lactate as a therapeutic agent would likely be tolerated well by human cells, as although non-wounded tissue in humans and rodents contain lactate at concentrations of 0.5–2 mM, wound levels can be at 5–15 mM or higher [142]. Lactate infusion studies in healthy patients which elevated blood plasma levels to 4 mM have also been conducted and shown a potential preference for lactate over glucose for brain cell metabolism [143].

Lactate's role in wound healing is one of particular interest to TBI- important in the regulation of VEGF and stimulating collagen deposition for formation of blood vessels and healing in cell culture models [142]. It was found that high lactate levels when accompanied by normoxic conditions, stimulated optimal blood vessel formation [142]. In subarachnoid haemorrhage (SAH), where blood vessel healing is critical, a microdialysis study in patients showed a pattern of elevated brain lactate and cerebral hyperglycolysis was associated with good recovery [144], while cerebral hypoxic lactate was associated with an increased mortality.

In early studies, Pellerin and Magistretti attributed brain lactate levels largely to astrocyte activity as blood-borne lactate does not easily cross the BBB and was therefore not a likely source in 'healthy' brain [24]. However, in brain perturbations such as TBI and SAH, periods of endogenous lactate import have been found to occur, which may help support injured brain [34,121,144].

In TBI, lactate infusions have been studied, not specifically for metabolic enhancement, but as an alternative for lowering intercranial pressure [145]. Metabolic inferences however have been made as patients who are initially more metabolically 'stressed' (i.e. an elevated LPR), had higher brain glucose concentrations after receiving hypertonic lactate infusion, than patients with lower

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

initial LPR [146]. This decreased glucose consumption indicates a preferential use of lactate in these elevated LPR cases, which could be beneficial for assisting energy production in more perturbed brains. The high lactate concentrations may also have a self-regulating effect, driving less lactate production by cells overall. This hyperosmolar nature of the infusion however could also be playing a key role in creating a more favourable environment for the brain and also reducing stress on brain cells [147]. Therefore, additional studies on non-hypertonic lactate would assist in better describing its individual role in these cases. This addition of lactate to the system would artificially increase the L/P ratio - a measure typically associated with worse outcome, and other biomarker measures would also have to be used for such studies.

5.3.4 Acetate

Acetate is another simple metabolite that is transported across the cellular membrane to be used in the TCA cycle and in the production of phospholipids. It is estimated that circulating acetate levels may contribute up to 10%–15% of the basal energy demands of astrocytes [148]. This increased acetate processing (also upregulated in tumour growth [149]) may be important for meeting the bioenergetic demands in TBI. In addition to acetate’s role as an energy substrate, it is noted to increase during stress, hypoxia exposure, and glucose deprivation. It has also been linked to HIF-2 signalling- required for lipid synthesis, proliferation, migration, and invasion in cancer cells *in vitro* [150].

Dichloroacetate (DCA) has been used to inhibit pyruvate dehydrogenase kinase. This decreases the conversion of pyruvate to lactate, promoting aerobic glycolysis in treatment of inherited mitochondrial disorders, pulmonary hypertension and solid tumours [151,152]. Several studies have been done on DCA and similar acetate-derived molecules to reduce lactate production *in vitro* [153,154], which may be applicable to TBI. Glyceryltriacetate (GTA), another form of bioavailable acetate, has similarly been used in rodent models of TBI, where it improved motor performance and increased ATP levels [155].

One difficulty with acetate supplementation in humans, however, is side effects such as alkalosis. Plasma acetate concentration in humans varies from 0.05 to 0.25 mM under resting conditions (and up to 1 mM after alcohol consumption), while the acetate concentration in mouse and rat plasma ranges from 0.20 to 0.30 mM, and as such rodents may tolerate supplementation better in pre-clinical studies [156]. Sodium acetate infusion in healthy human subjects at up to 2 mM in plasma produced a significant rise in plasma pyruvate, lactate, and α-hydroxybutyrate concentrations, indicating metabolic use, however this occurred along with temporary alkalosis [157]. High concentration daily infusions of DCA in humans was also associated with peripheral neuropathy [158,159].

Investigating the role of acetate in the metabolic and inflammatory response to TBI models could highlight another pathway in which we could attempt to regulate these processes, however its use as a supplement is currently limited in terms of concentration due to side-effects (see above).

5.3.5 Other metabolic supplementation pathways for consideration

Pyruvate is another candidate that has been clearly identified as a potential supplement for increasing metabolic substrate availability, which would effectively impact LPR. Pyruvate would also bypass the glycolysis step and potential diversion to lactate that can occur with glucose, however pyruvate may need to be given in a semi-altered form (e.g. ethyl pyruvate [160]) as pyruvate in solution can self-react, forming dimers (e.g. parapyruvate) that could inhibit the TCA cycle [161]. Ethyl pyruvate has been tested in pre-clinical rodent models of TBI, and has been found to improve cognitive function [162] and decrease neuronal loss [163], while sodium pyruvate can also decrease neuronal loss and attenuate metabolic dysfunction [164,165]. **HMGB1, an inflammatory protein which further increases release of cytokines, was also found to be reduced by ethyl pyruvate in a study of TBI in rats [166].** Human cell culture studies have also identified pyruvate as a possible therapeutic scavenger for free radicals created during NOS activity [167].

Ascorbic acid is another molecule warranting further investigation as a metabolic supplement, due to its potential role as a 'switch' in metabolic molecule uptake in neurons from glucose to lactate and ability to scavenge ROS [168,169].

The brain-gut axis is another pathway that is increasingly represented in the literature and may also need to be taken into consideration due its effects on inflammation and neurodegenerative disease [170]. Supplementing microbiota could have indirect effects on inflammation and metabolism. The brain-gut axis is outside the scope of this review, however is recognised in multiple sclerosis [171], and some preliminary studies in TBI [172,173].

5.4 Metabolic attenuation

As well as metabolic supplementation, metabolic attenuation could also have potential to have therapeutic effects on the post-TBI brain by regulating excessive and potentially harmful cell processes.

5.4.1 Glutamate

Glutamate is the most common neurotransmitter released by neurons for cell signalling and is linked to multiple cell processes. The astrocyte-neuron lactate

shuttle hypothesis links increased glutamate to stimulation of astrocyte metabolic pathways [24,138, 174], as previously described in Figure 2. In this hypothesis, astrocytes take up glutamate for processing, which also increases astrocytic glucose uptake.

In patients with TBI, increased glucose metabolism and LPR is associated with poorer outcomes, and increased glutamate may be contributing to this metabolic dysfunction via the ANLS pathway. Glutamate levels have been shown to rise in severe TBI cases, correlating with poorer outcomes [15]. **Glutamate transporters have also been found to be decreased in astrocytes following TBI [175].** Acute glutamate release is also associated with post-traumatic epilepsy and subsequent neuronal cell death, established in both rodent models [176,177], and in approximately 20% of human closed head injury patients [178]. In TBI patients, these epilepsy-associated electrophysiological disturbances are also associated with metabolic disturbances in terms of increased LPR [179]. **This increase in extracellular glutamate increases excitotoxicity [180] and further contributes to the brains pathological state.**

In addition to metabolism, **pathological glutamate release** is also linked to inflammatory pathways, as the local levels of this molecule have been associated with pro- or anti-inflammatory activation of cultured microglial cells [181]. This is hypothesised to act through glutamate's effect on the production of free radicals from nitric oxide synthase (NOS) activity [182].

Attenuating glutamate release to inhibit post-TBI damage and epilepsy is currently in early stages of investigation. Overexpression of the glutamate transporter, GLT-1, in mouse models significantly reduces ischemia-induced glutamate overflow, resulting in decreased cell death and improved recovery [183]. The impact of such experiments attenuating glutamate on the brain's metabolic status and potential inflammatory implications would also be of great interest. Similarly, the effect of any other supplementation on glutamate levels would be important to consider in clinical studies.

5.4.2 Cyclosporine

Cyclosporine is used in patients receiving transplants due to its immunosuppressive properties, however has since been explored further for drug repurposing. It was discovered to have additional metabolic functions, inhibiting mitochondrial membrane permeability and excessive ROS production. Cyclosporine was recently shown to exhibit these metabolic effects in pre-clinical **rat [184]** and porcine [185] models of TBI, reducing injury lesion volume and improving mitochondrial function. Previous studies in TBI patients have also found cyclosporine to decrease LPR [186]. **Cyclosporine's safety for use in TBI patients have been shown in phase II clinical**

data [187,188] with no significant difference in adverse effects compared to controls [189]. However, improved neuroprotection or cognitive outcome in humans is yet to be confirmed. This effect may be largely due to cyclosporine treatment corresponding to an increase in glucose levels detected in the microdialysates [162]. The combination of metabolic and anti-inflammatory actions marks cyclosporine a very noteworthy dual-acting therapy for future studies.

6. Conclusion and future potential: metabolic modulation as a dual-action anti-inflammatory therapy

There is a clearly emerging field of work highlighting the relationship between metabolism and inflammation with implications for many disease states. The diverging evidence for use of metabolic intermediates such as glucose and succinate as supplements highlights the importance of the disease context and brain environment in their application, and also their significant impact on brain homeostasis and patient outcome. Further studies of new metabolic intermediates supplements, or new drugs for attenuation of adverse metabolic tendencies, and how these candidates may alter inflammatory signalling are needed. This emerging field is of particular interest for their use in acute injury and recovery, as there is still no effective neuroprotective drug treatment for TBI. Investigation into these links and pathways are still in their early stages, however have great potential for new treatment avenues for further focus in neurological injury and disease.

7. Expert commentary

Understanding the nature of the abnormal cerebral metabolism following TBI, and its relationship with brain inflammation, will provide essential information for designing therapies. The results would have implications not only for neurocritical care but also for the long-term disability and accelerated onset of neurodegenerative diseases (e.g. dementia and Parkinson's) in post-TBI patients. Studies suggest that after the initial few days post-TBI there may still be an on-going and persistent inflammatory process. TBI patients show varying degrees of disability months or even years post-injury, and chronic low-grade brain inflammation may conceivably play a role. Mitochondrial function is known to play a key role in *in vitro* repolarization of such M1 cells to an M2 phenotype. Research in inflammation in TBI animal models has received considerable success and attention yet there is lack of evidence for benefit in human TBI patients. In general, TBI research suffers from a "failure to translate" whereby drugs that looked promising in animals have failed in clinical trials. Modern clinical technologies such as microdialysis and advanced scanning, in combination with laboratory-based instrumentation e.g. multiplex immuno-analysers for cytokine profiling and metabolic flux analysers for real-time measurements on cells, provide us with the means to further study TBI in patients, and should in turn inform design of

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

appropriate therapies. In particular, the possibility of improving mitochondrial function, promoting efficient oxidative metabolism, which in turn ameliorates brain inflammation, is an considerable therapeutic goal.

8. Five year view

The prospect of using metabolic supplementation to ameliorate secondary injury including inflammation after TBI merits further exploration in model systems, and in carefully controlled clinical studies in TBI and non-TBI individuals. Phase III studies of clinical outcomes are costly, and demand time, resources and large numbers of patients. It is therefore important that adequate Phase I and Phase II clinical studies are performed employing appropriate measurements and biomarkers. These include multiplex immunoassays, metabolic monitoring techniques such as microdialysis and ex-vivo analysis e.g. **ISCUSflex (a bedside clinical microdialysis analyser that performs enzymatic colorimetric assays for glucose, lactate, pyruvate, glutamate and glycerol)**, NMR, mass spectrometry, and in-vivo scanning (e.g. MRI and MRS).

Metabolic therapy for TBI patients is an exciting prospect for neurocritical care and for alleviating long-term disability and neurodegeneration in post-TBI patients. Moreover, metabolic therapy may also have potential for treating other conditions where evidence suggests that mitochondrial function is disrupted, including “ageing-related” diseases such as dementia and Parkinson’s, which often occur at an earlier age in brain injury survivors compared to those without any previous brain injury.

Key Issues

- Traumatic Brain Injury patients have no standard therapies to address the metabolic or inflammatory effects of their disease in the short or long term.
- Metabolic dysfunction- identified by a high lactate/pyruvate ratio despite adequate brain glucose and oxygenation, has been characterised as occurring in a subset of patients.
- Inflammatory system activation can continue for months or years post-TBI, needing both acute and long-term pharmacological interventions. Inflammation immediately post-TBI is difficult to manage due to complex pleotropic nature of the signalling molecules, cytokines and chemokines.
- Links between inflammatory cell actions and their metabolism have been found in cell culture models of neuronal and non-neuronal cells. The further study of these links in both single cell and ‘whole brain’ is needed to tease out the specific metabolism/inflammation pathways and links.
- Metabolic supplementation is a potential to impact on inflammation and solve the energy crisis in the brain. Glucose control is important for clinical outcome,

1
2
3 although strict control of such levels increase metabolic crisis. Succinate has
4 potential to ameliorate metabolic stress, however, only in adequately
5 oxygenated environments. Other supplementation candidates are also
6 emerging, yet further research is needed.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

Figure Legends

Figure 1. Schematic of glucose energetic metabolism pathways in the cell. Glycolysis, which produces two molecules of ATP per molecule of glucose, is followed by oxidative phosphorylation of pyruvate (in the mitochondrion, through the tricarboxylic acid (TCA) cycle and electron transport chain (ETC), which produces 34 molecules of ATP, or LDH processing of pyruvate to lactate, which produces no molecules of ATP. Diagram copyright © 2018 Monica J. Killen, reproduced here with her permission.

Figure 2. The astrocyte-neuron lactate shuttle (ANLS). Glucose is delivered via the brain vasculature and can be taken up by both neurons and astrocytes for processing. Both cell types are able to process this glucose to pyruvate, which can then be delivered for subsequent ATP generation in the mitochondria. Astrocytes, however, can also supplement neuronal energy production by converting pyruvate to lactate and transporting it to the neurons via monocarboxylate transporters (MCT). Neurons then process the lactate back to pyruvate for mitochondrial processing. This process is also linked to the recycling of synaptic glutamate and sodium uptake in astrocytes, which in turn stimulates the need for increased glucose import. Reproduced from [14]: Magistretti, P.J. and Allaman I., A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. Neuron, 2015. 86(4): p. 883-901. Copyright © 2015 Elsevier, reproduced with permission from Elsevier.

Figure 3. The phased cell-mediated response to acute brain injury. A complex inflammatory cascade develops through interaction between several physiological compartments [cerebrospinal fluid (CSF), brain parenchyma, and vascular]. The initial response is first triggered by signalling molecules known as danger-associated molecular patterns (DAMPs) which are released into the extracellular environment post-injury. This stimulates resident microglia and astrocytes to produce cytokines, chemokines, and other potent chemotactic substances which promote the recruitment of peripheral nervous system cells. Monocytes and neutrophils enter the brain parenchyma through the permeable blood–brain barrier (BBB), and can further intensify the cytotoxic environment with their own secretion of signalling proteins. An ongoing cytokine release initiates a long-term inflammatory process, where typically we see an increase in M2 ‘reparative’ cell phenotype, as opposed to the initial pro-inflammatory M1 activated cells. However, chronic inflammation and overactivation of this system can lead to injurious outcomes, such as epilepsy, depression, and auto-immunization toward brain-enriched antigens. Reproduced from [33]: Thelin, E.P., et al., Monitoring the Neuroinflammatory Response Following Acute Brain Injury. Frontiers in Neurology, 2017. 8: p. 351. Copyright © 2017 Thelin, Tajsic, Zeiler, Menon, Hutchinson, Carpenter, Morganti-Kossmann and Helmy, published open-access under a Creative Commons Attribution License (CC-BY).

Supplementary Table 1. Inflammatory and Metabolic Neurotherapeutics in TBI

Treatment	Effect	Author	Journal	Type	Cohort/Model	Outcome
Perispinal Etanercept (PSE)	TNF inhibition	Tobinick et al. 2012[106]	CNS Drugs, 26(12): p. 1051-1070.	Clinical	617 stroke, 12 TBI patients.	In the TBI cohort, motor impairment and spasticity were statistically significantly reduced. in the stroke group, improvements in motor impairment, spasticity, sensory impairment, cognition, psychological/ behavioural function, aphasia and pain.
Minocycline	Reduced inflammation and mitochondrial stabilisation. Inhibition of microglial activation and proliferation, reduced excitotoxicity, reduced neuronal and glial apoptosis, neutralization of oxygen radicals, nitric oxide synthase inhibition, metalloproteinase inhibition.	Scott et al. 2018 [103]	Brain, 141(2): p. 459-471.	Clinical	15 patients >6 months post-TBI	Reduces chronic microglial activation after brain trauma but increases markers of neurodegeneration.
		Casha et al. 2005 [102]	Brain, 135(4): p. 1224-1236.	Clinical	Spinal cord Injury. 27 minocycline, 25 placebo control	Greater motor recovery in cervical but not thoracic injury.
		Ng et al. 2012 [101]	Journal of Neurotrauma, 34(7): p. 1410-1425.	Pre-Clinical	Mouse- controlled cortical impact	Reduced microglial activation and promoted early neurological recovery.
Anakinra (recombinant IL-1ra)	Inhibit IL-1 β and IL-1 α function	Helmy et al. 2014 [50]	Journal of Cerebral Blood Flow & Metabolism, 34(5): p. 845-851.	Clinical	20 severe TBI patients (10 anakinra, 10 control)	Safety in patients, penetration of brain from initial subcutaneous injection. Difference in cytokine profiles, potential 'pro-inflammatory' effects.
		Newell et al. 2018 [108]	eNeuro, 5(2): p. 0385-17.2018.	Pre-Clinical	Mouse- fluid percussion injury	Improved learning/cognitive function.
Antibodies to IL-1 β and IL-1 α	Decreased IL-1 β and IL-1 α function	Lu et al. 2005 [107]	Journal of Neurotrauma, 22(8): p. 885-895.	Pre-Clinical	Rats- weight drop	Attenuated the TBI-induced loss of hippocampal neurons.
Ibuprofen	Antiplatelet effect, reversible cyclooxygenase inhibitor	Zangbar et al. 2015 [104]	The American Journal of Surgery, 209(6): p. 921-926.	Clinical	65 Ibuprofen, 130 control	No difference.
		Browne et al. 2006 [105]	Biochimica et Biophysica Acta - Molecular Cell Research, 1843(11): p. 2563-2582.	Pre-Clinical	Rat- fluid percussion injury	Worsened cognitive outcome, no effect on hippocampal and cortical tissue loss.
Cyclosporine	Preserve mitochondrial bioenergetic state, potential neuroprotective effect	Mazzeo et al. 2008 [186]	Acta Neurochirurgica, 150(10): p. 1019.	Clinical	50 severe TBI patients	Improved brain chemistry (lower LPR)

Glucose	Glycaemic control	Clayton et al. 2004 [127]	British Journal of Anaesthesia, 93(6): p. 761-767.	Clinical	391 head injured patients before, 452 patients after protocol implemented	Relative risk reduction in intensive care mortality with introduction of tight glycaemic control (blood glucose 4-7 mmol litre ⁻¹)
Succinate	Supplement mitochondrial Complex II activity	Jalloh et al. 2017 [123]	Journal of Cerebral Blood Flow & Metabolism, 37(7): p. 2626-2638.	Clinical	9 TBI patients	Improved brain chemistry (lower LPR)
		Stovell et al. 2018 [134]	Scientific Reports, 8(1): p. 11140.	Clinical	8 TBI patients	Improved brain chemistry (lower LPR, improved NADH/NAD ⁺ redox state, PCr/ATP ratio)
		Oguro et al. 2004 [135]	Internal Medicine, 43(5): p. 427-431.	Clinical	MELAS patient case study	Complete control of convulsions.
		Giorgi-Coll et al. 2017 [132]	Scientific Reports, 7(1): p. 1003.	Pre-Clinical	Rat cell culture	Improved metabolic performance (lower LPR, improved mitochondrial respiration)
Lactate	Metabolic supplement	Jalloh et al. 2018 [27]	Journal of Neurotrauma, 35(17): p. 2025-2035.	Clinical	9 severe TBI patients, 5 control	Lactate metabolised to pyruvate, before entering the TCA cycle, no difference between TBI and non-TBI metabolism.
Hypertonic Lactate	Reduce secondary intracranial hypertension, cerebral energetics	Quintard et al. 2016 [146]	Journal of Neurotrauma, 33(7): p. 681-687.	Clinical	24 severe TBI patients	Improvement of brain energetics.
		Ichai et al. 2009 [145]	Intensive Care Medicine, 35(3): p. 471-479.	Clinical	34 severe TBI patients	More effective reduction of ICP and better long-term outcome compared to mannitol.
Glyceryl triacetate (GTA)	Metabolic acetate supplementation	Arun et al. 2010 [155]	Journal of Neurotrauma, 27(1): p. 293-298.	Pre-Clinical	Rats- controlled cortical impact	increased the levels of both NAA and ATP and improved motor performance.
Sodium Pyruvate (SP) and Ethyl Pyruvate (EP)	Metabolic pyruvate supplementation	Moro et al. 2016 [164]	Brain Research, 1642(1): p. 270-277.	Pre-Clinical	Rats- controlled cortical impact	SP attenuates cerebral metabolic depression and both SP and EP decrease neuronal loss.
Ethyl Pyruvate	Neuroprotection via MMP-9 suppression and altered anti-inflammatory effects.	Shi et al. 2015 [162]	CNS Neuroscience and Therapeutics, 21(1): p.374-384.	Pre-Clinical	Rats- controlled cortical impact	Improved sensorimotor and cognitive functions, ameliorated brain tissue damage, attenuated BBB breach and brain edema.

References

1. Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: A global perspective. *Neurorehabilitation*, 22(5), 341-353 (2007).
2. Rubiano AM, Carney N, Chesnut R, Puyana JC. Global neurotrauma research challenges and opportunities. *Nature*, 527(7578), S193-197 (2015).
3. Dewan MC, Rattani A, Gupta S *et al.* Estimating the global incidence of traumatic brain injury. *Journal of Neurosurgery*, 1-18 (2018).
4. Stein DG. Embracing failure: What the Phase III progesterone studies can teach about TBI clinical trials. *Brain Inj*, 29(11), 1259-1272 (2015).
5. Howard RB, Sayeed I, Stein DG. Suboptimal Dosing Parameters as Possible Factors in the Negative Phase III Clinical Trials of Progesterone for Traumatic Brain Injury. *J Neurotrauma*, 34(11), 1915-1918 (2017).
6. McKee AC, Cantu RC, Nowinski CJ *et al.* Chronic Traumatic Encephalopathy in Athletes: Progressive Tauopathy After Repetitive Head Injury. *Journal of Neuropathology & Experimental Neurology*, 68(7), 709-735 (2009).
7. McKee AC, Daneshvar DH. The neuropathology of traumatic brain injury. *Handbook of clinical neurology*, 127, 45-66 (2015).
8. Smith DH, Johnson VE, Stewart W. Chronic neuropathologies of single and repetitive TBI: substrates of dementia? *Nature reviews. Neurology*, 9(4), 211-221 (2013).
9. Minambres E, Cemborain A Fau - Sanchez-Velasco P, Sanchez-Velasco P Fau - Gandarillas M *et al.* Correlation between transcranial interleukin-6 gradient and outcome in patients with acute brain injury. (0090-3493 (Print)) (2003).
10. Selwyn R, Hockenbury N, Jaiswal S, Mathur S, Armstrong RC, Byrnes KR. Mild Traumatic Brain Injury Results in Depressed Cerebral Glucose Uptake: An 18FDG PET Study. *Journal of Neurotrauma*, 30(23), 1943-1953 (2013).
11. Helmy A, Carpenter KL, Menon DK, Pickard JD, Hutchinson PJ. The cytokine response to human traumatic brain injury: temporal profiles and evidence for cerebral parenchymal production. *J Cereb Blood Flow Metab*, 31(2), 658-670 (2011).
12. Ramlackhansingh AF, Brooks DJ, Greenwood RJ *et al.* Inflammation after trauma: microglial activation and traumatic brain injury. *Ann Neurol*, 70(3), 374-383 (2011).

*** This study demonstrates that after TBI, increased microglial activation can be present for many years. TBI triggers a chronic inflammatory response particularly in subcortical regions. It is important to considering the response to TBI as evolving over time and interventions may be beneficial for longer intervals after trauma than previously assumed.**

13. Balan IS, Saladino AJ, Aarabi B *et al.* Cellular Alterations in Human Traumatic Brain Injury: Changes in Mitochondrial Morphology Reflect Regional Levels of Injury Severity. *Journal of Neurotrauma*, 30(5), 367-381 (2013).
14. Nordstrom CH, Nielsen TH, Schalen W, Reinstrup P, Ungerstedt U. Biochemical indications of cerebral ischaemia and mitochondrial dysfunction in severe brain trauma analysed with regard to type of lesion. *Acta Neurochir (Wien)*, 158(7), 1231-1240 (2016).
15. Timofeev I, Carpenter KL, Nortje J *et al.* Cerebral extracellular chemistry and outcome following traumatic brain injury: a microdialysis study of 223 patients. *Brain*, 134(Pt 2), 484-494 (2011).

*** In the largest study of microdialysis monitoring in TBI patients published to date, high lactate/pyruvate ratio was statistically associated with worse patient outcome.**

16. Peruzzotti-Jametti L, Pluchino S. Targeting Mitochondrial Metabolism in Neuroinflammation: Towards a Therapy for Progressive Multiple Sclerosis. *Trends in Molecular Medicine*, 24(10), 838-855 (2018).
17. Sliter DA, Martinez J, Hao L *et al*. Parkin and PINK1 mitigate STING-induced inflammation. *Nature*, (2018).
18. Yin F, Sancheti H, Patil I, Cadenas E. Energy metabolism and inflammation in brain aging and Alzheimer's disease. *Free radical biology & medicine*, 100, 108-122 (2016).
19. De Felice F, Lourenco M. Brain metabolic stress and neuroinflammation at the basis of cognitive impairment in Alzheimer's disease. *Frontiers in Aging Neuroscience*, 7, 94 (2015).
20. Appel SH, Zhao W, Beers DR, Henkel JS. The microglial-motoneuron dialogue in ALS. *Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology*, 30(1), 4-8 (2011).
21. Ganeshan K, Chawla A. Metabolic regulation of immune responses. *Annu Rev Immunol*, 32, 609-634 (2014).
22. Lodish H BA, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. *Molecular Cell Biology* (W. H. Freeman, New York, 2000).
23. Berg JM JM, Tymoczko JL. *Biochemistry* (W.H. Freeman, New York, 2002).
24. Pellerin L, Pellegrini G, Bittar PG *et al*. Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev Neurosci*, 20(4-5), 291-299 (1998).

*** Evidence was found for astrocytes producing lactate and transporting it to neurons for further energetic use.**

25. Pellerin L, Magistretti PJ. Sweet sixteen for ANLS. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 32(7), 1152-1166 (2012).
26. Magistretti Pierre J, Allaman I. A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. *Neuron*, 86(4), 883-901 (2015).
27. Jalloh I, Helmy A, Howe DJ *et al*. A Comparison of Oxidative Lactate Metabolism in Traumatically Injured Brain and Control Brain. *Journal of neurotrauma*, 35(17), 2025-2035 (2018).
28. Mächler P, Wyss Matthias T, Elsayed M *et al*. In vivo Evidence for a Lactate Gradient from Astrocytes to Neurons. *Cell Metabolism*, 23(1), 94-102 (2016).
29. Waagepetersen HS, Bakken IJ, Larsson OM, Sonnewald U, Schousboe A. Comparison of Lactate and Glucose Metabolism in Cultured Neocortical Neurons and Astrocytes Using ¹³C-NMR Spectroscopy. *Developmental Neuroscience*, 20(4-5), 310-320 (1998).
30. Boumezbeur F, Petersen KF, Cline GW *et al*. The Contribution of Blood Lactate to Brain Energy Metabolism in Humans Measured by Dynamic (13)C Nuclear Magnetic Resonance Spectroscopy. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(42), 13983-13991 (2010).
31. Patel AB, Lai JCK, Chowdhury GMI *et al*. Direct evidence for activity-dependent glucose phosphorylation in neurons with implications for the astrocyte-to-neuron lactate shuttle. *Proceedings of the National Academy of Sciences*, 111(14), 5385 (2014).
32. Carpenter KL, Jalloh I, Gallagher CN *et al*. (13)C-labelled microdialysis studies of cerebral metabolism in TBI patients. *Eur J Pharm Sci*, 57, 87-97 (2014).
33. Sarrafzadeh AS, Kiening KL, Callsen TA, Unterberg AW. Metabolic changes during impending and manifest cerebral hypoxia in traumatic brain injury. *British Journal of Neurosurgery*, 17(4), 340-346 (2003).
34. Jalloh I, Carpenter KLH, Grice P *et al*. Glycolysis and the pentose phosphate pathway after human traumatic brain injury: microdialysis studies using 1,2-(13)C(2) glucose. *Journal of Cerebral Blood Flow & Metabolism*, 35(1), 111-120 (2015).

35. Di Pietro V, Lazzarino G, Amorini AM *et al.* Fusion or Fission: The Destiny of Mitochondria In Traumatic Brain Injury of Different Severities. *Scientific Reports*, 7(1), 9189 (2017).
36. Cheng G, Kong R-h, Zhang L-m, Zhang J-n. Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies. *British Journal of Pharmacology*, 167(4), 699-719 (2012).
37. Swerdlow RH, Parks JK, Miller SW *et al.* Origin and functional consequences of the complex I defect in Parkinson's disease. *Annals of Neurology*, 40(4), 663-671 (1996).
38. Lazzarino G, Amorini AM, Petzold A *et al.* Serum Compounds of Energy Metabolism Impairment Are Related to Disability, Disease Course and Neuroimaging in Multiple Sclerosis. *Molecular Neurobiology*, 54(9), 7520-7533 (2017).
39. Bowling AC, Schulz JB, Brown RH, Jr., Beal MF. Superoxide Dismutase Activity, Oxidative Damage, and Mitochondrial Energy Metabolism in Familial and Sporadic Amyotrophic Lateral Sclerosis. *Journal of Neurochemistry*, 61(6), 2322-2325 (1993).
40. Davey GP, Peuchen S, Clark JB. Energy thresholds in brain mitochondria: Potential involvement in neurodegeneration. *Journal of Biological Chemistry*, 273(21), 12753-12757 (1998).
41. Petrosillo G, Matera M, Moro N, Ruggiero FM, Paradies G. Mitochondrial complex I dysfunction in rat heart with aging: critical role of reactive oxygen species and cardiolipin. *Free Radical Biology and Medicine*, 46(1), 88-94 (2009).
42. Ventura B, Genova ML, Bovina C, Formiggini G, Lenaz G. Control of oxidative phosphorylation by Complex I in rat liver mitochondria: implications for aging. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1553(3), 249-260 (2002).
43. Fang EF, Scheibye-Knudsen M, Chua KF, Mattson MP, Croteau DL, Bohr VA. Nuclear DNA damage signalling to mitochondria in ageing. *Nature Reviews Molecular Cell Biology*, 17, 308 (2016).
44. Carpenter KL, Jalloh I, Hutchinson PJ. Glycolysis and the significance of lactate in traumatic brain injury. *Front Neurosci*, 9, 112 (2015).
45. Liesz A, Dalpke A, Mracsko E *et al.* DAMP Signaling is a Key Pathway Inducing Immune Modulation after Brain Injury. *The Journal of Neuroscience*, 35(2), 583 (2015).
46. Zhang Q, Raoof M, Chen Y *et al.* Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*, 464, 104 (2010).
47. Lenzlinger PM, Morganti-Kossmann M-C, Laurer HL, McIntosh TK. The duality of the inflammatory response to traumatic brain injury. *Molecular Neurobiology*, 24(1), 169-181 (2001).
48. Hinson HE, Rowell S, Schreiber M. Clinical evidence of inflammation driving secondary brain injury: A systematic review. *Journal of Trauma and Acute Care Surgery*, 78(1) (2015).
49. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain*, 136(1), 28-42 (2013).
50. Helmy A, Guilfoyle MR, Carpenter KL, Pickard JD, Menon DK, Hutchinson PJ. Recombinant human interleukin-1 receptor antagonist in severe traumatic brain injury: a phase II randomized control trial. *J Cereb Blood Flow Metab*, 34(5), 845-851 (2014).
51. Thelin EP, Tajsic T, Zeiler FA *et al.* Monitoring the Neuroinflammatory Response Following Acute Brain Injury. *Frontiers in Neurology*, 8, 351 (2017).
52. Bellander B-M, Singhrao SK, Ohlsson M, Mattsson P, Svensson M. Complement Activation in the Human Brain after Traumatic Head Injury. *Journal of Neurotrauma*, 18(12), 1295-1311 (2001).
53. Schäfer MKH, Schwaebler WJ, Post C *et al.* Complement C1q Is Dramatically Up-Regulated in Brain Microglia in Response to Transient Global Cerebral Ischemia. *The Journal of Immunology*, 164(10), 5446 (2000).
54. Hammad A, Westacott L, Zaben M. The role of the complement system in traumatic brain injury: a review. *Journal of neuroinflammation*, 15(1), 24-24 (2018).

55. Vink R, Gabrielian L, Thornton E. The Role of Substance P in Secondary Pathophysiology after Traumatic Brain Injury. *Frontiers in Neurology*, 8, 304 (2017).
56. Tanaka R, Komine-Kobayashi M, Mochizuki H *et al.* Migration of enhanced green fluorescent protein expressing bone marrow-derived microglia/macrophage into the mouse brain following permanent focal ischemia. *Neuroscience*, 117(3), 531-539 (2003).
57. Schilling M, Besselmann M, Müller M, Strecker JK, Ringelstein EB, Kiefer R. Predominant phagocytic activity of resident microglia over hematogenous macrophages following transient focal cerebral ischemia: An investigation using green fluorescent protein transgenic bone marrow chimeric mice. *Experimental Neurology*, 196(2), 290-297 (2005).
58. Adam D, Rishma V, Jianghua F *et al.* Proliferating Resident Microglia after Focal Cerebral Ischaemia in Mice. *Journal of Cerebral Blood Flow & Metabolism*, 27(12), 1941-1953 (2007).
59. Herisson F, Frodermann V, Courties G *et al.* Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration. *Nature Neuroscience*, 21(9), 1209-1217 (2018).
60. Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF- α , TGF- β 1 and blood-brain barrier function. *Journal of Neuroimmunology*, 101(2), 211-221.
61. Semple BD, Bye N, Ziebell JM, Morganti-Kossmann MC. Deficiency of the chemokine receptor CXCR2 attenuates neutrophil infiltration and cortical damage following closed head injury. *Neurobiology of Disease*, 40(2), 394-403 (2010).
62. Farooqui AA, Horrocks LA, Farooqui T. Modulation of inflammation in brain: a matter of fat. *J Neurochem*, 101(3), 577-599 (2007).
63. Ziebell JM, Morganti-Kossmann MC. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics*, 7(1), 22-30 (2010).
64. Becher B, Spath S, Goverman J. Cytokine networks in neuroinflammation. *Nat Rev Immunol*, 17(1), 49-59 (2017).
65. Cantaert T, Baeten D, Tak PP, van Baarsen LGM. Type I IFN and TNF α cross-regulation in immune-mediated inflammatory disease: basic concepts and clinical relevance. *Arthritis Research & Therapy*, 12(5), 219 (2010).
66. Salim T, Sershen CL, May EE. Investigating the Role of TNF- α and IFN- γ Activation on the Dynamics of iNOS Gene Expression in LPS Stimulated Macrophages. *PLOS ONE*, 11(6), e0153289 (2016).
67. Singhal A, Baker AJ, Hare GMT, Reinders FX, Schlichter LC, Moulton RJ. Association between Cerebrospinal Fluid Interleukin-6 Concentrations and Outcome after Severe Human Traumatic Brain Injury. *Journal of Neurotrauma*, 19(8), 929-937 (2002).
68. Italiani P, Boraschi D. From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. *Front Immunol*, 5, 514 (2014).
69. Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? *Nature Neuroscience*, 19, 987 (2016).
70. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Reports*, 6, 13 (2014).
71. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *The Journal of Clinical Investigation*, 122(3), 787-795 (2012).
72. Guillemin GJ, Brew BJ. Microglia, macrophages, perivascular macrophages, and pericytes: A review of function and identification. *Journal of Leukocyte Biology*, 75(3), 388-397 (2004).
73. Ford AL, Goodsall AL, Hickey WF, Sedgwick JD. Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting. Phenotypic differences defined and direct ex vivo antigen presentation to myelin basic

protein-reactive CD4+ T cells compared. *The Journal of Immunology*, 154(9), 4309 (1995).

74. Satoh J-i, Kino Y, Asahina N *et al.* TMEM119 marks a subset of microglia in the human brain. *Neuropathology*, 36(1), 39-49 (2015).
75. Bennett ML, Bennett FC, Liddel SA *et al.* New tools for studying microglia in the mouse and human CNS. *Proceedings of the National Academy of Sciences of the United States of America*, 113(12), E1738-E1746 (2016).
76. Perry VH, Teeling J. Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Seminars in Immunopathology*, 35(5), 601-612 (2013).
77. Trahanas DM, Cuda CM, Perlman H, Schwulst SJ. Differential activation of infiltrating monocyte-derived cells after mild and severe traumatic brain injury. *Shock (Augusta, Ga.)*, 43(3), 255-260 (2015).
78. Girard S, Brough D, Lopez-Castejon G, Giles J, Rothwell NJ, Allan SM. Microglia and Macrophages Differentially Modulate Cell Death After Brain Injury Caused by Oxygen-Glucose Deprivation in Organotypic Brain Slices. *Glia*, 61(5), 813-824 (2013).
79. Liddel SA, Guttenplan KA, Clarke LE *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*, 541(7638), 481-487 (2017).
80. Hovda DA, Gurkoff GG, Sofroniew MV, Lee SM, Myer DJ. Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain*, 129(10), 2761-2772 (2006).
81. Mierzwa AJ, Sullivan GM, Armstrong RC, Marion CM, McDaniel DP. Components of Myelin Damage and Repair in the Progression of White Matter Pathology After Mild Traumatic Brain Injury. *Journal of Neuropathology & Experimental Neurology*, 74(3), 218-232 (2015).
82. Armstrong RC, Mierzwa AJ, Marion CM, Sullivan GM. White matter involvement after TBI: Clues to axon and myelin repair capacity. *Experimental Neurology*, 275, 328-333 (2016).
83. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med*, 213(1), 15-23 (2016).

**** M1 and M2 phenotypes can be induced by metabolic stress. Metabolic re-programming is recognized as being among other key immunoregulatory events that govern the nature of the immune response, both in health and disease.**

84. Rodriguez-Prados JC, Traves PG, Cuenca J *et al.* Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J Immunol*, 185(1), 605-614 (2010).
85. Vats D, Mukundan L, Odegaard JI *et al.* Oxidative metabolism and PGC-1 β attenuate macrophage-mediated inflammation. *Cell Metab*, 4(1), 13-24 (2006).
86. Gao F, Chen D, Hu Q, Wang G. Rotenone directly induces BV2 cell activation via the p38 MAPK pathway. *PLoS One*, 8(8), e72046 (2013).
87. Ye J, Jiang Z, Chen X, Liu M, Li J, Liu N. Electron transport chain inhibitors induce microglia activation through enhancing mitochondrial reactive oxygen species production. *Exp Cell Res*, 340(2), 315-326 (2016).
88. Mount MP, Lira A, Grimes D *et al.* Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. *J Neurosci*, 27(12), 3328-3337 (2007).
89. Gao H-M, Hong J-S, Zhang W, Liu B. Distinct Role for Microglia in Rotenone-Induced Degeneration of Dopaminergic Neurons. *The Journal of Neuroscience*, 22, 782-790 (2002).
90. Emmrich JV, Hornik TC, Neher JJ, Brown GC. Rotenone induces neuronal death by microglial phagocytosis of neurons. *FEBS J*, 280(20), 5030-5038 (2013).
91. Sheng W, Zong Y, Mohammad A *et al.* Pro-inflammatory cytokines and lipopolysaccharide induce changes in cell morphology, and upregulation of ERK1/2, iNOS and sPLA(2)-IIA expression in astrocytes and microglia. *Journal of Neuroinflammation*, 8, 121-121 (2011).

92. Littlewood-Evans A, Sarret S, Apfel V *et al.* GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. *J Exp Med*, 213(9), 1655-1662 (2016).
93. Sheth SA, Iavarone AT, Liebeskind DS, Won SJ, Swanson RA. Targeted Lipid Profiling Discovers Plasma Biomarkers of Acute Brain Injury. *PLoS One*, 10(6), e0129735 (2015).
94. Thelin EP, Zeiler FA, Ercole A *et al.* Serial Sampling of Serum Protein Biomarkers for Monitoring Human Traumatic Brain Injury Dynamics: A Systematic Review. *Frontiers in Neurology*, 8, 300 (2017).
95. CRASH_Trial_collaborators. Effect of intravenous corticosteroids on death within 14 days in 10 008 adults with clinically significant head injury (MRC CRASH trial): randomised placebo-controlled trial. *The Lancet*, 364(9442), 1321-1328 (2004).
96. Wright DW, Yeatts SD, Silbergleit R *et al.* Very Early Administration of Progesterone for Acute Traumatic Brain Injury. *New England Journal of Medicine*, 371(26), 2457-2466 (2014).
97. Skolnick BE, Maas AI, Narayan RK *et al.* A Clinical Trial of Progesterone for Severe Traumatic Brain Injury. *New England Journal of Medicine*, 371(26), 2467-2476 (2014).
98. Zafonte RD, Bagiella E, Ansel BM, *et al.* Effect of citicoline on functional and cognitive status among patients with traumatic brain injury: Citicoline brain injury treatment trial (cobrit). *JAMA*, 308(19), 1993-2000 (2012).
99. Temkin NR, Anderson GD, Winn HR *et al.* Magnesium sulfate for neuroprotection after traumatic brain injury: a randomised controlled trial. *The Lancet Neurology*, 6(1), 29-38 (2007).
100. Robertson CS, McCarthy JJ, Miller ER *et al.* Phase II Clinical Trial of Atorvastatin in Mild Traumatic Brain Injury. *Journal of Neurotrauma*, 34(7), 1394-1401 (2016).
101. Ng SY, Semple BD, Morganti-Kossmann MC, Bye N. Attenuation of microglial activation with minocycline is not associated with changes in neurogenesis after focal traumatic brain injury in adult mice. *Journal of Neurotrauma*, 29(7), 1410-1425 (2012).
102. Casha S, Zygun D, McGowan MD, Bains I, Yong VW, John Hurlbert R. Results of a phase II placebo-controlled randomized trial of minocycline in acute spinal cord injury. *Brain*, 135(4), 1224-1236 (2012).
103. Scott G, Zetterberg H, Jolly A *et al.* Minocycline reduces chronic microglial activation after brain trauma but increases neurodegeneration. *Brain : a journal of neurology*, 141(2), 459-471 (2018).
104. Zangbar B, Pandit V, Rhee P *et al.* Clinical outcomes in patients on preinjury ibuprofen with traumatic brain injury. *The American Journal of Surgery*, 209(6), 921-926 (2015).
105. Browne KD, Iwata A, Putt ME, Smith DH. Chronic ibuprofen administration worsens cognitive outcome following traumatic brain injury in rats. *Experimental Neurology*, 201(2), 301-307 (2006).
106. Tobinick E, Kim NM, Reyzin G, Rodriguez-Romanacce H, Depuy V. Selective TNF inhibition for chronic stroke and traumatic brain injury: An observational study involving 629 consecutive patients treated with perispinal etanercept. *CNS Drugs*, 26(12), 1051-1070 (2012).
107. Lu K-T, Wang Y-W, Yang J-T, Yang Y-L, Chen H-I. Effect of Interleukin-1 on Traumatic Brain Injury-Induced Damage to Hippocampal Neurons. *Journal of Neurotrauma*, 22(8), 885-895 (2005).
108. Newell EA, Todd BP, Mahoney J, Pieper AA, Ferguson PJ, Bassuk AG. Combined Blockade of Interleukin-1 α and -1 β Signaling Protects Mice from Cognitive Dysfunction after Traumatic Brain Injury. *eNeuro*, 5(2), ENEURO.0385-0317.2018 (2018).
109. James G, Kayode O, Sharon H *et al.* Reduction of inflammation after administration of interleukin-1 receptor antagonist following aneurysmal subarachnoid hemorrhage: results of the Subcutaneous Interleukin-1Ra in SAH (SCIL-SAH) study. *Journal of Neurosurgery JNS*, 128(2), 515-523 (2018).

110. Hutchinson PJ, O'Connell MT, Rothwell NJ *et al.* Inflammation in Human Brain Injury: Intracerebral Concentrations of IL-1 α , IL-1 β , and Their Endogenous Inhibitor IL-1ra. *Journal of Neurotrauma*, 24(10), 1545-1557 (2007).
111. Helmy A, Guilfoyle MR, Carpenter KLH, Pickard JD, Menon DK, Hutchinson PJ. Recombinant human interleukin-1 receptor antagonist promotes M1 microglia biased cytokines and chemokines following human traumatic brain injury. *Journal of Cerebral Blood Flow and Metabolism*, 36(8), 1434-1448 (2016).
112. Hellewell S, Semple BD, Morganti-Kossmann MC. Therapies negating neuroinflammation after brain trauma. *Brain Research*, 1640, 36-56 (2016).
113. Bergold PJ. Treatment of traumatic brain injury with anti-inflammatory drugs. *Experimental Neurology*, 275, 367-380 (2016).
114. Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: Opportunities for therapeutic intervention. *Brain, Behavior, and Immunity*, 26(8), 1191-1201 (2012).
115. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1843(11), 2563-2582 (2014).
116. Gaba A, Grivennikov SI, Do MV, Stumpo DJ, Blackshear PJ, Karin M. Cutting Edge: IL-10-Mediated Tristetraprolin Induction Is Part of a Feedback Loop That Controls Macrophage STAT3 Activation and Cytokine Production. *The Journal of Immunology*, (2012).
117. Graham DB, Jasso GJ, Mok A *et al.* Nitric Oxide Engages an Anti-inflammatory Feedback Loop Mediated by Peroxiredoxin 5 in Phagocytes. *Cell Reports*, 24(4), 838-850 (2018).
118. Stovell MG, Mada MO, Carpenter TA *et al.* Phosphorus spectroscopy in acute TBI demonstrates metabolic changes that relate to outcome in the presence of normal structural MRI. *Journal of Cerebral Blood Flow & Metabolism*, 0271678X18799176 (2018).
119. Oresic M, Posti JP, Kamstrup-Nielsen MH *et al.* Human Serum Metabolites Associate With Severity and Patient Outcomes in Traumatic Brain Injury. *EBioMedicine*, 12, 118-126 (2016).
120. Yi L, Shi S, Wang Y *et al.* Serum Metabolic Profiling Reveals Altered Metabolic Pathways in Patients with Post-traumatic Cognitive Impairments. *Scientific Reports*, 6, 21320 (2016).
121. Glenn TC, Hirt D, Mendez G *et al.* Metabolomic Analysis of Cerebral Spinal Fluid from Patients with Severe Brain Injury. In: *Brain Edema XV*. Katayama, Y, Maeda, T, Kuroiwa, T (Eds) (Springer Vienna, Vienna, 2013) 115-119.
122. Gallagher CN, Carpenter KLH, Grice P *et al.* The human brain utilizes lactate via the tricarboxylic acid cycle: a ^{13}C -labelled microdialysis and high-resolution nuclear magnetic resonance study. *Brain*, 132(10), 2839-2849 (2009).
123. Jalloh I, Helmy A, Howe DJ *et al.* Focally perfused succinate potentiates brain metabolism in head injury patients. *Journal of Cerebral Blood Flow & Metabolism*, 37(7), 2626-2638 (2017).
- * **Direct tricarboxylic acid cycle supplementation with 2,3- $^{13}\text{C}_2$ succinate, delivered by microdialysis, improved human TBI brain chemistry, indicated by biomarkers and ^{13}C -labelling patterns in metabolites in the microdialysates, suggesting succinate as a potential therapy.**
124. Young B, Ott L, Dempsey R, Haack D, Tibbs P. Relationship between admission hyperglycemia and neurologic outcome of severely brain-injured patients. *Annals of Surgery*, 210(4), 466-473 (1989).
125. Vespa PM, McArthur D, O'Phelan K *et al.* Persistently Low Extracellular Glucose Correlates with Poor Outcome 6 Months after Human Traumatic Brain Injury despite a Lack of Increased Lactate: A Microdialysis Study. *Journal of Cerebral Blood Flow & Metabolism*, 23(7), 865-877 (2003).

126. Meierhans R, Béchir M, Ludwig S *et al.* Brain metabolism is significantly impaired at blood glucose below 6 mM and brain glucose below 1 mM in patients with severe traumatic brain injury. *Critical Care*, 14(1), R13-R13 (2010).
127. Clayton TJ, Nelson RJ, Manara AR. Reduction in mortality from severe head injury following introduction of a protocol for intensive care management†‡. *British Journal of Anaesthesia*, 93(6), 761-767 (2004).
128. Hermanides J, Plummer MP, Finnis M, Deane AM, Coles JP, Menon DK. Glycaemic control targets after traumatic brain injury: a systematic review and meta-analysis. *Critical Care*, 22, 11 (2018).
129. Vespa P, McArthur DL, Stein N *et al.* Tight glycemic control increases metabolic distress in traumatic brain injury: A randomized controlled within-subjects trial*. *Critical Care Medicine*, 40(6) (2012).

*** The findings of this study importantly suggest that in neurocritical care of TBI patients, control of blood glucose levels should not be too tight, and that more liberal glucose control is better.**

130. Plummer MP, Notkina N, Timofeev I, Hutchinson PJ, Finnis ME, Gupta AK. Cerebral metabolic effects of strict versus conventional glycaemic targets following severe traumatic brain injury. *Critical Care*, 22, 16 (2018).
131. Tannahill GM, Curtis AM, Adamik J *et al.* Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature*, 496, 238 (2013).
132. Giorgi-Coll S, Amaral AI, Hutchinson PJA, Kotter MR, Carpenter KLH. Succinate supplementation improves metabolic performance of mixed glial cell cultures with mitochondrial dysfunction. *Sci Rep*, 7(1), 1003 (2017).
133. Chouchani ET, Pell VR, Gaude E *et al.* Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*, 515(7527), 431-435 (2014).
134. Stovell MG, Mada MO, Helmy A *et al.* The effect of succinate on brain NADH/NAD⁺ redox state and high energy phosphate metabolism in acute traumatic brain injury. *Scientific Reports*, 8(1), 11140 (2018).
135. Oguro H, Iijima K, Takahashi K *et al.* Successful Treatment with Succinate in a Patient with MELAS. *Internal Medicine*, 43(5), 427-431 (2004).
136. Mills EL, Kelly B, Logan A *et al.* Repurposing mitochondria from ATP production to ROS generation drives a pro-inflammatory phenotype in macrophages that depends on succinate oxidation by complex II. *Cell*, 167(2), 457-470.e413 (2016).
137. Peruzzotti-Jametti L, Bernstock JD, Vicario N *et al.* Macrophage-Derived Extracellular Succinate Licenses Neural Stem Cells to Suppress Chronic Neuroinflammation. *Cell Stem Cell*, 22(3), 355-368.e313 (2018).
138. Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A*, 91(22), 10625-10629 (1994).
139. Prieto R, Tavazzi B, Taya K *et al.* Brain energy depletion in a rodent model of diffuse traumatic brain injury is not prevented with administration of sodium lactate. *Brain Research*, 1404, 39-49 (2011).
140. Immke DC, McCleskey EW. Lactate enhances the acid-sensing Na⁺ channel on ischemia-sensing neurons. *Nature Neuroscience*, 4, 869 (2001).
141. Margineanu MB, Mahmood H, Fiumelli H, Magistretti PJ. L-Lactate Regulates the Expression of Synaptic Plasticity and Neuroprotection Genes in Cortical Neurons: A Transcriptome Analysis. *Frontiers in Molecular Neuroscience*, 11, 375 (2018).
142. Constant JS, Feng JJ, Zabel DD *et al.* Lactate elicits vascular endothelial growth factor from macrophages: a possible alternative to hypoxia. *Wound Repair and Regeneration*, 8(5), 353-360 (2000).

143. Diarmuid S, Andrew P, William AH, Emma B, Paul KM, Stephanie AA. Lactate: A Preferred Fuel for Human Brain Metabolism in Vivo. *Journal of Cerebral Blood Flow & Metabolism*, 23(6), 658-664 (2003).
144. Oddo M, Levine JM, Frangos S *et al.* Brain Lactate Metabolism in Humans With Subarachnoid Hemorrhage. *Stroke*, 43(5), 1418 (2012).
145. Ichai C, Armando G, Orban J-C *et al.* Sodium lactate versus mannitol in the treatment of intracranial hypertensive episodes in severe traumatic brain-injured patients. *Intensive Care Medicine*, 35(3), 471-479 (2009).
146. Quintard H, Patet C, Zerlauth J-B *et al.* Improvement of Neuroenergetics by Hypertonic Lactate Therapy in Patients with Traumatic Brain Injury Is Dependent on Baseline Cerebral Lactate/Pyruvate Ratio. *Journal of Neurotrauma*, 33(7), 681-687 (2016).
147. Francony G FB, Falcon D, Canet C, Dilou H, Lavagne P, Jacquot C, Payen JF. Equimolar doses of mannitol and hypertonic saline in the treatment of increased intracranial pressure. *Critical Care Medicine*, 36(3), 795-800 (2008).
148. Mashimo T, Pichumani K, Vemireddy V *et al.* Acetate Is a Bioenergetic Substrate for Human Glioblastoma and Brain Metastases. *Cell*, 159(7), 1603-1614 (2014).
149. Chen R, Xu M, Nagati JS *et al.* The Acetate/ACSS2 Switch Regulates HIF-2 Stress Signaling in the Tumor Cell Microenvironment. *PLOS ONE*, 10(2), e0116515 (2015).
150. Gao X, Lin S-H, Ren F *et al.* Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. *Nature Communications*, 7, 11960 (2016).
151. Sanchez WY, McGee SL, Connor T *et al.* Dichloroacetate inhibits aerobic glycolysis in multiple myeloma cells and increases sensitivity to bortezomib. *Br J Cancer*, 108(8), 1624-1633 (2013).
152. James MO, Jahn SC, Zhong G, Smeltz MG, Hu Z, Stacpoole PW. Therapeutic applications of dichloroacetate and the role of glutathione transferase zeta-1. *Pharmacol Ther*, 170, 166-180 (2017).
153. Schmidt MM, Rohwedder A, Dringen R. Effects of chlorinated acetates on the glutathione metabolism and on glycolysis of cultured astrocytes. *Neurotox Res*, 19(4), 628-637 (2011).
154. Dimlich RVW, Marangos PJ. Dichloroacetate attenuates neuronal damage in a gerbil model of brain ischemia. *Journal of Molecular Neuroscience*, 5(2), 69-81 (1994).
155. Arun P, Ariyannur PS, Moffett JR *et al.* Metabolic Acetate Therapy for the Treatment of Traumatic Brain Injury. *Journal of Neurotrauma*, 27(1), 293-298 (2009).
156. Schug Zachary T, Peck B, Jones Dylan T *et al.* Acetyl-CoA Synthetase 2 Promotes Acetate Utilization and Maintains Cancer Cell Growth under Metabolic Stress. *Cancer Cell*, 27(1), 57-71 (2015).
157. Richards RH, Vreman HJ, Zager P, Feldman C, Blaschke T, Weiner MW. Acetate Metabolism in Normal Human Subjects. *American Journal of Kidney Diseases*, 2(1), 47-57 (1982).
158. Kaufmann P, Engelstad K, Wei Y *et al.* Dichloroacetate causes toxic neuropathy in MELAS. *Neurology*, 66(3), 324 (2006).
159. Stacpoole PW, Henderson GN, Yan Z, Cornett R, James MO. Pharmacokinetics, Metabolism, and Toxicology of Dichloroacetate. *Drug Metabolism Reviews*, 30(3), 499-539 (1998).
160. Fink MP. Ethyl pyruvate. *Current Opinion in Anesthesiology*, 21(2) (2008).
161. Margolis SA, Coxon B. Identification and quantitation of the impurities in sodium pyruvate. *Analytical Chemistry*, 58(12), 2504-2510 (1986).
162. Shi H, Wang HL, Pu HJ *et al.* Ethyl pyruvate protects against blood-brain barrier damage and improves long-term neurological outcomes in a rat model of traumatic brain injury. *CNS Neurosci Ther*, 21 (2015).
163. Su X, Wang H, Zhu L, Zhao J, Pan H, Ji X. Ethyl pyruvate ameliorates intracerebral hemorrhage-induced brain injury through anti-cell death and anti-inflammatory mechanisms. *Neuroscience*, 245 (2013).

164. Moro N, Ghavim SS, Harris NG, Hovda DA, Sutton RL. Pyruvate treatment attenuates cerebral metabolic depression and neuronal loss after traumatic experimental brain injury. *Brain Res*, 1642 (2016).
165. Moro N, Sutton RL. Beneficial effects of sodium or ethyl pyruvate after traumatic brain injury in the rat. *Exp Neurol*, 225 (2010).
166. Su X, Wang H, Zhao J, Pan H, Mao L. Beneficial effects of ethyl pyruvate through inhibiting high-mobility group box 1 expression and TLR4/NFkB pathway after traumatic brain injury in the rat. *Mediators Inflamm*, 2011 (2011).
167. Wang X, Perez E, Liu R, Yan L-J, Mallet RT, Yang S-H. Pyruvate Protects Mitochondria from Oxidative Stress in Human Neuroblastoma SK-N-SH Cells. *Brain research*, 1132(1), 1-9 (2007).
168. Castro MA, Beltrán FA, Brauchi S, Concha II. A metabolic switch in brain: glucose and lactate metabolism modulation by ascorbic acid. *Journal of Neurochemistry*, 110(2), 423-440 (2009).
169. Covarrubias-Pinto A, Acuña AI, Beltrán FA, Torres-Díaz L, Castro MA. Old Things New View: Ascorbic Acid Protects the Brain in Neurodegenerative Disorders. *International Journal of Molecular Sciences*, 16(12), 28194-28217 (2015).
170. Round JL, Mazmanian SK. The gut microbiome shapes intestinal immune responses during health and disease. *Nature reviews. Immunology*, 9(5), 313-323 (2009).
171. Dopkins N, Nagarkatti PS, Nagarkatti M. The role of gut microbiome and associated metabolome in the regulation of neuroinflammation in multiple sclerosis and its implications in attenuating chronic inflammation in other inflammatory and autoimmune disorders. *Immunology*, 154(2), 178-185 (2018).
172. Sundman MH, Chen N-k, Subbian V, Chou Y-h. The bidirectional gut-brain-microbiota axis as a potential nexus between traumatic brain injury, inflammation, and disease. *Brain, Behavior, and Immunity*, 66, 31-44 (2017).
173. Waligora-Dupriet A-J, Lafleur S, Charrueau C *et al.* Head injury profoundly affects gut microbiota homeostasis: Results of a pilot study. *Nutrition*, 45, 104-107 (2018).
174. Mason S. Lactate Shuttles in Neuroenergetics-Homeostasis, Allostasis and Beyond. *Front Neurosci*, 11, 43 (2017).
175. Landeghem FKHV, Weiss T, Oehmichen M, Deimling AV. Decreased Expression of Glutamate Transporters in Astrocytes after Human Traumatic Brain Injury. *Journal of Neurotrauma*, 23(10), 1518-1528 (2006).
176. Cantu D, Walker K, Andresen L *et al.* Traumatic Brain Injury Increases Cortical Glutamate Network Activity by Compromising GABAergic Control. *Cerebral Cortex (New York, NY)*, 25(8), 2306-2320 (2015).
177. Amorini AM, Lazzarino G, Di Pietro V *et al.* Severity of experimental traumatic brain injury modulates changes in concentrations of cerebral free amino acids. *Journal of cellular and molecular medicine*, 21(3), 530-542 (2017).
178. Guerriero RM, Giza CC, Rotenberg A. Glutamate and GABA imbalance following traumatic brain injury. *Current neurology and neuroscience reports*, 15(5), 27-27 (2015).
179. Vespa P, Tubi M, Claassen J *et al.* Metabolic crisis occurs with seizures and periodic discharges after brain trauma. *Annals of Neurology*, 79(4), 579-590 (2016).
180. Yi J-H, Hazell AS. Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. *Neurochemistry International*, 48(5), 394-403 (2006).
181. Barger SW, Goodwin ME, Porter MM, Beggs ML. Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *Journal of neurochemistry*, 101(5), 1205-1213 (2007).
182. Dai S-S, Zhou Y-G, Li W *et al.* Local Glutamate Level Dictates Adenosine A(2A) Receptor Regulation of Neuroinflammation and Traumatic Brain Injury. *The Journal of Neuroscience*, 30(16), 5802-5810 (2010).
183. Harvey BK, Airavaara M, Hinzman J *et al.* Targeted Over-Expression of Glutamate Transporter 1 (GLT-1) Reduces Ischemic Brain Injury in a Rat Model of Stroke. *PLOS ONE*, 6(8), e22135 (2011).

- 1
2
3 184. Signoretti S, Marmarou A, Tavazzi B *et al.* The Protective Effect of Cyclosporin A upon
4 N-Acetylaspartate and Mitochondrial Dysfunction following Experimental Diffuse
5 Traumatic Brain Injury. *Journal of Neurotrauma*, 21(9), 1154-1167 (2004).
6
7 185. Karlsson M, Pukenas B, Chawla S *et al.* Neuroprotective Effects of Cyclosporine in a
8 Porcine Pre-Clinical Trial of Focal Traumatic Brain Injury. *Journal of Neurotrauma*,
9 (2018).
10 186. Mazzeo AT, Alves ÓL, Gilman CB *et al.* Brain metabolic and hemodynamic effects of
11 cyclosporin A after human severe traumatic brain injury: a microdialysis study. *Acta*
12 *Neurochirurgica*, 150(10), 1019 (2008).
13

14 **** The immunosuppressant cyclosporine also produces metabolic effects in TBI**
15 **patients, such as improved LPR, which may be due to corresponding significant effects**
16 **on glucose levels.**
17

- 18 187. Jimmi H, Bonnie R, Philip E, Richard K, Byron Y. Dosing and safety of cyclosporine in
19 patients with severe brain injury. *Journal of Neurosurgery JNS*, 109(4), 699-707 (2008).
20 188. Mazzeo AT, Brophy GM, Gilman CB *et al.* Safety and Tolerability of Cyclosporin A in
21 Severe Traumatic Brain Injury Patients: Results from a Prospective Randomized Trial.
22 *Journal of Neurotrauma*, 26(12), 2195-2206 (2009).
23 189. Aminmansour B, Fard SA, Habibabadi MR, Moein P, Norouzi R, Naderan M. The
24 efficacy of Cyclosporine-A on Diffuse Axonal Injury after Traumatic Brain Injury.
25 *Advanced biomedical research*, 3, 35-35 (2014).
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

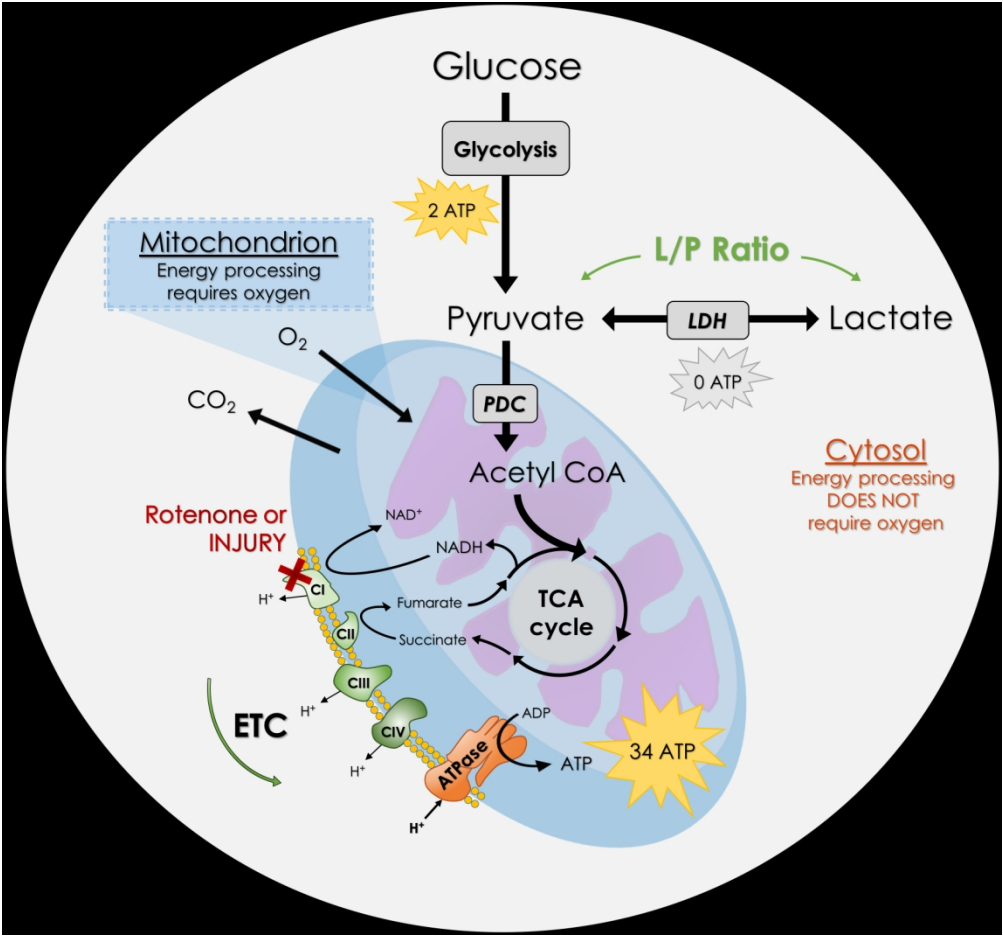


Figure 1. Schematic of glucose energetic metabolism pathways in the cell. Glycolysis, which produces two molecules of ATP per molecule of glucose, is followed by oxidative phosphorylation of pyruvate (in the mitochondrion, through the tricarboxylic acid (TCA) cycle and electron transport chain (ETC), which produces 34 molecules of ATP, or LDH processing of pyruvate to lactate, which produces no molecules of ATP. Diagram copyright © 2018 Monica Killen, reproduced here with her permission.

301x280mm (150 x 150 DPI)

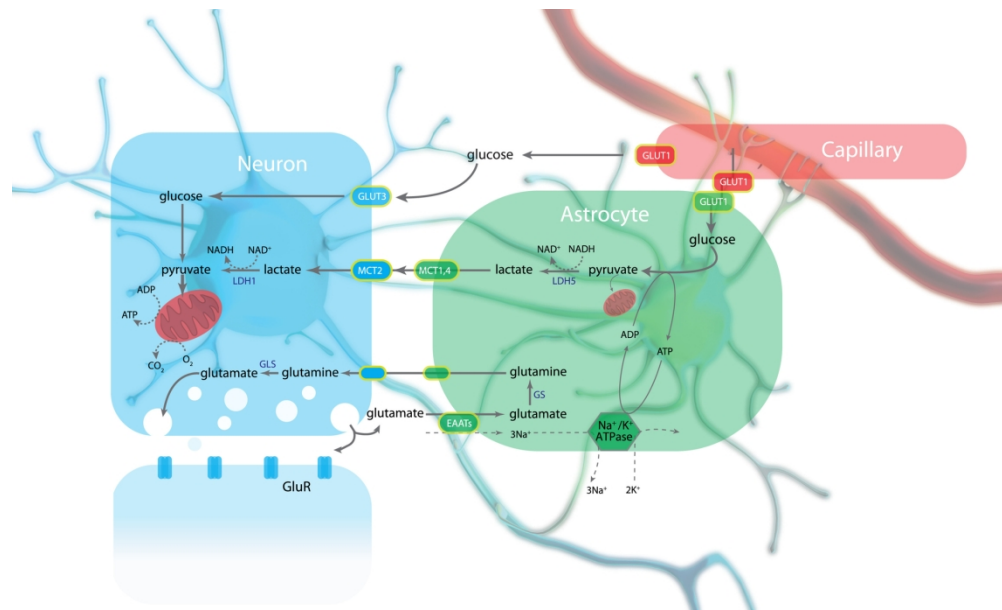


Figure 2. The astrocyte-neuron lactate shuttle (ANLS). Glucose is delivered via the brain vasculature and can be taken up by both neurons and astrocytes for processing. Both cell types are able to process this glucose to pyruvate, which can then be delivered for subsequent ATP generation in the mitochondria. Astrocytes, however, can also supplement neuronal energy production by converting pyruvate to lactate and transporting it to the neurons via monocarboxylate transporters (MCT). Neurons then process the lactate back to pyruvate for mitochondrial processing. This process is also linked to the recycling of synaptic glutamate and sodium uptake in astrocytes, which in turn stimulates the need for increased glucose import. Reproduced from [14]: Magistretti, P.J. and Allaman I., A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. *Neuron*, 2015. 86(4): p. 883-901. DOI 10.1016/j.neuron.2015.03.035, Copyright © 2015 Elsevier, reproduced with permission from Elsevier (CC-BY-NC-ND).

172x103mm (300 x 300 DPI)

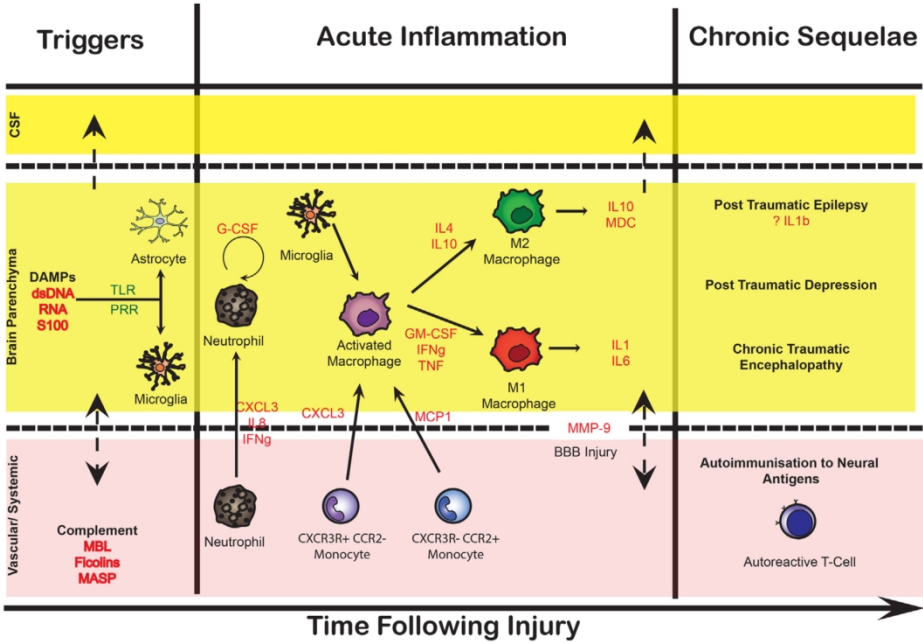


Figure 3. The phased cell-mediated response to acute brain injury. A complex inflammatory cascade develops through interaction between several physiological compartments [cerebrospinal fluid (CSF), brain parenchyma, and vascular]. The initial response is first triggered by signalling molecules known as danger-associated molecular patterns (DAMPs) which are released into the extracellular environment post-injury. This stimulates resident microglia and astrocytes to produce cytokines, chemokines, and other potent chemotactic substances which promote the recruitment of peripheral nervous system cells. Monocytes and neutrophils enter the brain parenchyma through the permeable blood–brain barrier (BBB), and can further intensify the cytotoxic environment with their own secretion of signalling proteins. An ongoing cytokine release initiates a long-term inflammatory process, where typically we see an increase in M2 ‘reparative’ cell phenotype, as opposed to the initial pro-inflammatory M1 activated cells. However, chronic inflammation and overactivation of this system can lead to injurious outcomes, such as epilepsy, depression, and auto-immunization toward brain-enriched antigens. Reproduced from [33]: Thelin, E.P., et al., Monitoring the Neuroinflammatory Response Following Acute Brain Injury. *Frontiers in Neurology*, 2017. 8: p. 351. Copyright © 2017 Thelin, Tajsic, Zeiler, Menon, Hutchinson, Carpenter, Morganti-Kossmann and Helmy, published open-access under a Creative Commons Attribution License (CC-BY).

358x234mm (144 x 144 DPI)

Supplementary Table 1. Inflammatory and Metabolic Neurotherapeutics in TBI

Treatment	Effect	Author	Journal	Type	Cohort/Model	Outcome
Perispinal Etanercept (PSE)	TNF inhibition	Tobinick et al. 2012 [106]	CNS Drugs, 26(12): p. 1051-1070.	Clinical	617 stroke, 12 TBI patients.	In the TBI cohort, motor impairment and spasticity were statistically significantly reduced. In the stroke group, improvements in motor impairment, spasticity, sensory impairment, cognition, psychological/behavioural function, aphasia and pain.
Minocycline	Reduced inflammation and mitochondrial stabilisation. Inhibition of microglial activation and proliferation, reduced excitotoxicity, reduced neuronal and glial apoptosis, neutralization of oxygen radicals, nitric oxide synthase inhibition, metalloproteinase inhibition.	Scott et al. 2018 [103]	Brain, 141(2): p. 459-471.	Clinical	15 patients >6 months post-TBI	Reduces chronic microglial activation after brain trauma but increases markers of neurodegeneration.
		Casha et al. 2005 [102]	Brain, 135(4): p. 1224-1236.	Clinical	Spinal cord Injury. 27 minocycline, 25 placebo control	Greater motor recovery in cervical but not thoracic injury.
		Ng et al. 2012 [101]	Journal of Neurotrauma, 34(7): p. 1410-1425.	Pre-Clinical	Mouse- controlled cortical impact	Reduced microglial activation and promoted early neurological recovery.
Anakinra (recombinant IL-1ra)	Inhibit IL-1 β and IL-1 α function	Helmy et al. 2014 [50]	Journal of Cerebral Blood Flow & Metabolism, 34(5): p. 845-851.	Clinical	20 severe TBI patients (10 anakinra, 10 control)	Safety in patients, penetration of brain from initial subcutaneous injection. Difference in cytokine profiles, potential 'pro-inflammatory' effects.
		Newell et al. 2018 [108]	eNeuro, 5(2): p. 0385-17.2018.	Pre-Clinical	Mouse- fluid percussion injury	Improved learning/cognitive function.
Antibodies to IL-1 β and IL-1 α	Decreased IL-1 β and IL-1 α function	Lu et al. 2005 [107]	Journal of Neurotrauma, 22(8): p. 885-895.	Pre-Clinical	Rats- weight drop	Attenuated the TBI-induced loss of hippocampal neurons.
Ibuprofen	Antiplatelet effect, reversible cyclooxygenase inhibitor	Zangbar et al. 2015 [104]	The American Journal of Surgery, 209(6): p. 921-926.	Clinical	65 Ibuprofen, 130 control	No difference.
		Browne et al. 2006 [105]	Biochimica et Biophysica Acta - Molecular Cell Research, 1843(11): p. 2563-2582.	Pre-Clinical	Rat- fluid percussion injury	Worsened cognitive outcome, no effect on hippocampal and cortical tissue loss.
Cyclosporine	Preserve mitochondrial bioenergetic state, potential neuroprotective effect	Mazzeo et al. 2008 [186]	Acta Neurochirurgica, 150(10): p. 1019.	Clinical	50 severe TBI patients	Improved brain chemistry (lower LPR)

Glucose	Glycaemic control	Clayton et al. 2004 [127]	British Journal of Anaesthesia, 93(6): p. 761-767.	Clinical	391 head injured patients before, 452 patients after protocol implemented	Relative risk reduction in intensive care mortality with introduction of tight glycaemic control (blood glucose 4-7 mmol litre ⁻¹)
Succinate	Supplement mitochondrial Complex II activity	Jalloh et al. 2017 [123]	Journal of Cerebral Blood Flow & Metabolism, 37(7): p. 2626-2638.	Clinical	9 TBI patients	Improved brain chemistry (lower LPR)
		Stovell et al. 2018 [134]	Scientific Reports, 8(1): p. 11140.	Clinical	8 TBI patients	Improved brain chemistry (lower LPR, improved NADH/NAD ⁺ redox state, PCr/ATP ratio)
		Oguro et al. 2004 [135]	Internal Medicine, 43(5): p. 427-431.	Clinical	MELAS patient case study	Complete control of convulsions.
		Giorgi-Coll et al. 2017 [132]	Scientific Reports, 7(1): p. 1003.	Pre-Clinical	Rat cell culture	Improved metabolic performance (lower LPR, improved mitochondrial respiration)
Lactate	Metabolic supplement	Jalloh et al. 2018 [27]	Journal of Neurotrauma, 35(17): p. 2025-2035.	Clinical	9 severe TBI patients, 5 control	Lactate metabolised to pyruvate, before entering the TCA cycle, no difference between TBI and non-TBI metabolism.
Hypertonic Lactate	Reduce secondary intracranial hypertension, cerebral energetics	Quintard et al. 2016 [146]	Journal of Neurotrauma, 33(7): p. 681-687.	Clinical	24 severe TBI patients	Improvement of brain energetics.
		Ichai et al. 2009 [145]	Intensive Care Medicine, 35(3): p. 471-479.	Clinical	34 severe TBI patients	More effective reduction of ICP and better long-term outcome compared to mannitol.
Glyceryl triacetate (GTA)	Metabolic acetate supplementation	Arun et al. 2010 [155]	Journal of Neurotrauma, 27(1): p. 293-298.	Pre-Clinical	Rats- controlled cortical impact	increased the levels of both NAA and ATP and improved motor performance.
Sodium Pyruvate (SP) and Ethyl Pyruvate (EP)	Metabolic pyruvate supplementation	Moro et al. 2016 [164]	Brain Research, 1642(1): p. 270-277.	Pre-Clinical	Rats- controlled cortical impact	SP attenuates cerebral metabolic depression and both SP and EP decrease neuronal loss.
Ethyl Pyruvate	Neuroprotection via MMP-9 suppression and altered anti-inflammatory effects.	Shi et al. 2015 [162]	CNS Neuroscience and Therapeutics, 21(1): p.374-384.	Pre-Clinical	Rats- controlled cortical impact	Improved sensorimotor and cognitive functions, ameliorated brain tissue damage, attenuated BBB breach and brain edema.